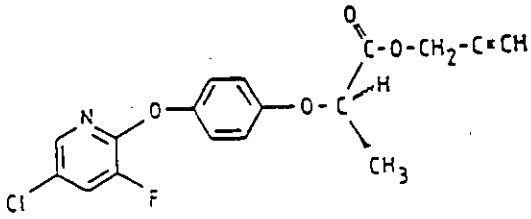
Determination of Residues of Metabolites CGA 193469 and CGA 153433 by Liquid Chromatography (HPLC)	**SOIL**	Mar. 19, 1992 PP 2.53, PM

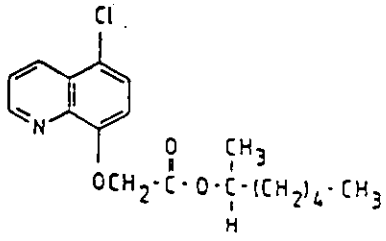
TABLE OF CONTENTS

CHEMICAL STRUCTURES

PARENT COMPOUNDS
(not analyzed by this method)

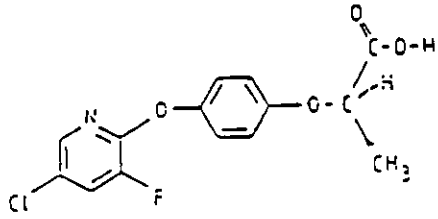


CGA 184927

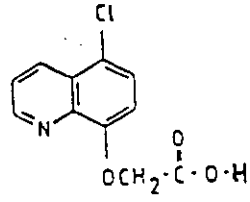


CGA 185072

METABOLITES



CGA 193469



CGA 153433

1. INTRODUCTION

1.1 Scope of the Method

CGA 184927, an experimental herbicide, is applied together with the safener CGA 185072 to crop and weeds post-emergence. The major metabolites of CGA 184927 and CGA 185072 in plant and soil metabolism appear to be CGA 193469 and CGA 153433, resp.

This method allows residues of metabolites CGA 193469 and CGA 153433 to be quantitated in ***soil*** at a ***lower practical level*** of ***0.005 mg/kg.***

1.2 Principle of the Method

Homogenized soil specimens are extracted with a mixture of acetone and buffer pH 3. After evaporation of the organic solvent, CGA 193469 is extracted into hexane-diethyl ether at acidic pH, while CGA 153433 is afterwards extracted into dichloro methane from the same aqueous extract. The two organic solutions are evaporated, separately taken up in ion pair reagent solution and cleaned up by extraction with hexane.

The metabolites are then independently determined by reversed-phase HPLC with UV-detection.

2. MATERIALS AND METHODS

Standard laboratory equipment is not listed. All equipment and chemicals mentioned herein can be substituted by suitable products of any origin. Prove suitability of reagents by analyzing reagent blanks.

2.1 Equipment

2.1.1 Sample concentrator DB-3 with heating block (Techne Ltd., Duxford, Cambridge England)

2.1.2 Laboratory Shaker, Type KL-2 (E. Bühler, Tübingen, FRG)

2.1.3 Labor Centrifuge "Mistral 2000" (MSE Scientific Instruments, Crawley, West Sussex Great Britain)

2.1.4 Syringe Filters Acrodisc LC13 PVDF 0.45 µm (Gelman, Ann Arbor, MI USA)

2.2 Reagents

Main suppliers' addresses:
- Fluka Chemie AG, CH-9470 Buchs.
- E. Merck AG, D-6100 Darmstadt.

2.2.1 Acetone, for residue analysis, (Merck, # 12)

2.2.2 Acetonitrile, HPLC grade, (Fluka, # 692)

2.2.3 Dichloro methane, analytical grade, (Merck, # 6050)

2.2.4 Diethyl ether, analytical grade, (Merck, # 921)

2.2.5 n-Hexane, for residue analysis, (Merck, # 4371)

2.2.6 Water, HPLC grade, (prepared in house)

2.2.7 Ion pair reagent concentrate: PIC A Low UV reagent (Waters part# 84189, contains 1.7 g (5 mmol) tetrabutyl ammonium dihydrogen phosphate) in approx. 20 ml water

2.2.8 Citric acid monohydrate, (Merck # 244)

2.2.9 Sodium chloride, analytical grade, (Merck # 6404)

2.2.10 Sodium hydroxide solution, 1 N, (prepared in house)

2.2.11 Citrate buffer pH 3 (0.2 M), made from 8.4 g 2.2.8, 3.5 g 2.2.9, 21 mL 2.2.10 and water up to 200 mL.

2.2.12 CGA 193469 reference substance for standardization and recovery tests
Prepare a stock solution of CGA 193469 (200 µg/mL) in acetonitrile.

2.2.13 CGA 153433 reference substance for standardization and recovery tests
Prepare a stock solution of CGA 153433 (100 µg/mL due to low solubility) in acetonitrile.

2.3 Analytical Procedure

2.3.1 Preparation of Specimens and Subspecimens

Homogenize the whole specimen sufficiently to allow variances of replicate analysis of subspecimens not to exceed the variance of the chromatographic system as estimated from the standard injections. Since the recommended subspecimen size of this method (2 g) is very small, prove suitability of the homogenization procedure by performing some replicate analysis of specimens containing residues. Store the specimens at freezer temperature until analysis.

For analysis weigh a 2 g subspecimen into a 30 ml glass centrifuge tube. If correction of residue results for moisture content of soil has to be performed, either correct each subspecimen size appropriately or introduce the correction factors in the calculation procedure.

2.3.2 Fortification

To regularly check the performance of the method, analyze also at least two fortified control specimens with each series of analyses. To prepare these specimens, add for each fortification level known amounts of CGA 193469 and CGA 153433 to the same uncontaminated control specimen prior to extraction. Select the fortification levels to be either two- and ten- to twenty-times the lower practical level of determination, or in the range of the expected residues. Prepare a fortification solution of CGA 193469 and CGA 153433 at a concentration of 1 µg/mL each by appropriate dilution of the stock solutions (see sections 2.2.12 and 2.2.13) with buffer pH = 3 (0.04 M, from diluting 0.2 M buffer with water 1 vol + 4 vol).

0.5 mg/kg level

Fortify control subspecimens by adding 5 µL and 10 µL of the respective stock solutions with a calibrated syringe. This fortification level has been included in method development work, to prove the suitability of the method for higher residue levels.

0.05 mg/kg level

Fortify control subspecimens by adding 100 µL of the fortification solution with a calibrated syringe.

0.01 mg/kg level

Fortify control subspecimens by adding 20 µL of the fortification solution with a calibrated syringe.

2.3.3 Extraction

Add 25 mL of a mixture of acetone (2 vol) and buffer (0.2 M) pH 3 (1 vol) to the soil subspecimen (section 2.3.1). Shake for 1 min at 300 cycles/min. Centrifuge for 5 min at 1500 rpm. Transfer the supernatant to a 50 mL graduated cylinder and repeat extraction step a second time, *shaking this time for 5 min.* Bring the combined supernatant solution to 50 mL with acetone and transfer an aliquot of 25 mL to a graduated test tube. Evaporate on the sample concentrator down to a volume of approx. 10 mL using a gentle stream of air at 45 °C heater block temperature.

2.3.4 Extraction of CGA 193469 into Organic Solvent

Add 4 mL of hexane-diethyl ether (8 vol + 2 vol) to the concentrated extract solution and shake thoroughly. Transfer the supernatant organic phase - containing CGA 193469 - to a 10 mL test tube. Repeat the extraction step once with 4 mL of the hexane-diethyl ether mixture and combine the organic phases in the test tube.

Evaporate the solvent to dryness in a gentle stream of air using the sample concentrator at ~45 °C block temperature. Redisolve the residue in 2 mL aqueous ion pair reagent solution (1 bottle PIC A per L of water).

Save aqueous phase for extraction of CGA 153433 (see 2.3.5).

2.3.5 Extraction of CGA 153433 into Organic Solvent

Add 8 mL of dichloro methane to the aqueous phase from step 2.3.4. and shake thoroughly. Transfer the supernatant organic phase - containing CGA 153433 - to a 25 mL test tube. Repeat the extraction step once with 8 mL of dichloro methane and combine the organic phases in the test tube. Evaporate the solvent to dryness in a gentle stream of air using the sample concentrator at ~45 °C block temperature. Redisolve the residue in 2 mL aqueous ion pair reagent solution (1 bottle of PIC A per L of water).

2.3.6. Cleanup of Analyte Solutions by Extraction with Hexane

Separately add 5 ml hexane to the aqueous solutions from steps 2.3.4 and 2.3.5 containing the analytes and shake. Remove organic layer with a Pasteur pipette and discard. Repeat extractions with another 5 mL portion of hexane. Discard organic phases. Filter aqueous phases through Gelman Acrodisc LC PVDF and inject into HPLC system.

2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic System

For determination of CGA 193469 and CGA 153433 use HPLC two-column-switching systems with UV-detector, pump, and autosampler-injector as follows (or with suitable equivalents).

Detector:	UV/VIS Detector UVIS 204 (Linear Instruments, Reno NV, USA)
Pump:	Dual piston pump model LC-9A (SHIMADZU, Kyoto, Japan)
Autosampler:	Automatic Sampling System PROMIS (SPARK, 7800 AJ Emmen Holland)
Recorder:	Dual channel recorder SE 120 (ABB Goerz AG, Wien, Austria);sensitivity set to 10 mV full scale; chart speed: 0.5 cm/min
Column 1:	Stainless steel, 25 cm length, 4 mm id.; packing: Nucleosil-100 C-18, particle size 7 µm (Macherey&Nagel, Düren, FRG)
Column 2:	Stainless steel, 25 cm length, 4.6 mm id.; packing: Inertsil-100 ODS, particle size 5 µm (VDS Optilab GmbH, Berlin, FRG)

Optional (for system automation): control and data collection unit, HP 3350 Laboratory Automation System, Hewlett-Packard, Avondale, PA 19311, USA

Chromatographic Conditions for Determination of CGA 193469:

Mobile phase 1: 320 mL Acetonitrile + 680 mL Water + 1 bottle of PIC-A ion pair solution at flow rate of 0.85 mL/min

Mobile phase 2: 350 mL Acetonitrile + 650 mL Water + 1 bottle of PIC-A ion pair solution at flow rate of 1.0 mL/min

Detector wave length: 226 nm

Detector sensitivity: 0.004 aufs

Volume injected: 500 µL

Retention time: ~23 min (depending on conditions chosen)

Chromatographic Conditions for Determination of CGA 153433:

Mobile phase 1: 230 mL Acetonitrile + 770 mL Water + 1 bottle of PIC-A ion pair solution at flow rate of 0.9 mL/min

Mobile phase 2: 270 mL Acetonitrile + 730 mL Water + 1 bottle of PIC-A ion pair solution at flow rate of 1.1 mL/min

Detector wave length: 244 nm

Detector sensitivity: 0.004 aufs

Volume injected: 200 µL

Retention time: ~29 min (depending on conditions chosen)

2.4.2 Calibration of the Chromatographic System by External Standards

Standardize the chromatographic system each time a series of specimens is to be quantitated. The range of the concentrations is depending on the range of residues to be determined, in particular, the lowest standard concentration is depending on the lower practical level.

For preparation of standard solutions calculate the lowest standard concentration (C) as follows:

$$C = \frac{L \times W \times V_a}{V_e \times V_f} [\mu g/mL]$$

L is lower practical level (mg/kg)
W is weight of subspecimen (g)
V_a is volume of aliquot cleaned up (mL)
V_e is volume of extract solution (mL)
V_f is final volume for determination (mL)

Example: C = 0.005 x 2 x 25 / (50 x 2) = 0.0025 µg/mL.

Prepare at least four standard solutions of different concentrations by diluting the stock solutions with aqueous ion pair reagent solution (1 bottle of PIC A per L of water). Evaporate the solvent of the stock solution prior to dilution (in air stream at ~40 °C heater temperature). Select the concentrations as required. Typical values are: 0.0025, 0.005, 0.01, 0.025, 0.05 µg/mL.

Since detector response may change on injections of coextractives, inject final and standard solutions alternatingly.

2.5 Interferences

None known so far

2.6 Confirmatory Techniques

None developed.

2.7 Time Required for Analysis

A series of 10 specimens can be processed during two working days. Automated HPLC chromatographic analysis can be performed overnight.

2.8 Modifications and Potential Problems

Interferences may originate from reagents used (e.g. ion pair reagent). In case of difficulties, run "reagent blanks" through the method for locating the cause of troubles.

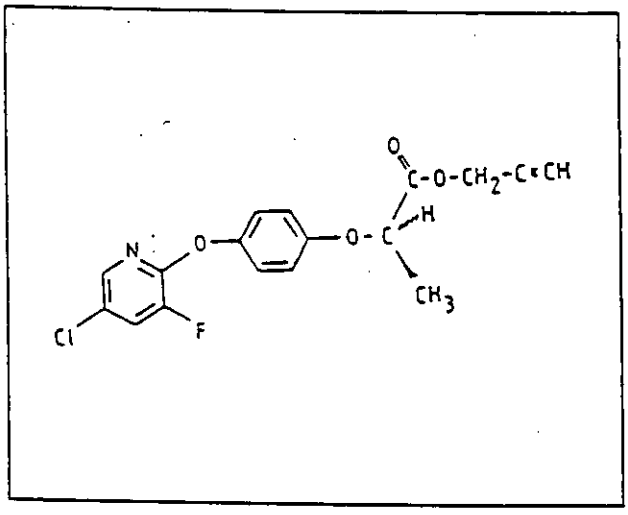
2.9 Calculation Procedure

Standardize the chromatographic system as outlined in section 2.4. Measure the response of the analytes at the characteristic retention time. Refer to REM 119.04 (reference 5.1) for the detailed description of the recommended calculation procedure. Calculate a correction factor for residues found, recoveries, and LPL according to the moisture content of soil specimens, if necessary. Alternatively, corrected subspecimen sizes can be used (see also 2.3.1)

6. APPENDICES

6.1 Figures

Figure 1: Structure and Chemical Name of CGA 184927

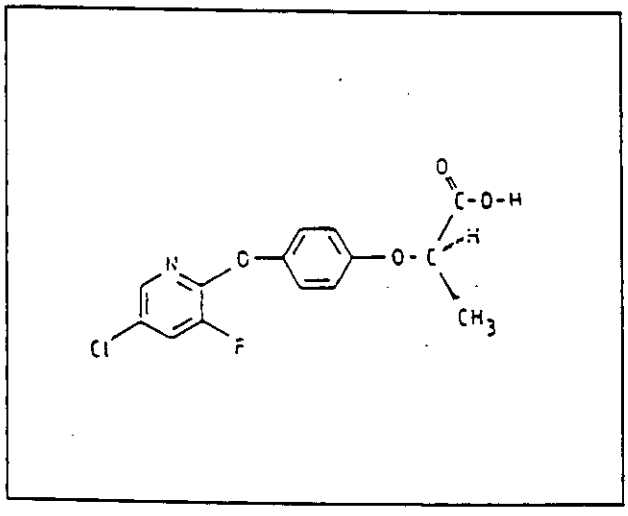


$C_{17}H_{13}ClFNO_4$

molecular mass: 349.7 amu

2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propanoic acid-2-propynylester

Figure 2: Structure and Chemical Name of Metabolite CGA 193469.

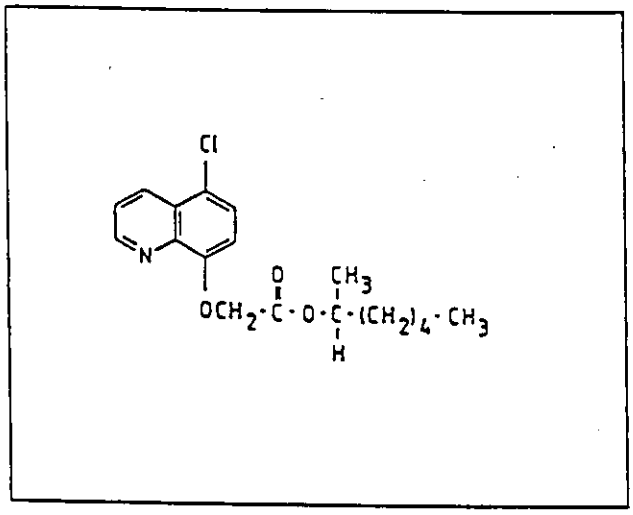


$C_{14}H_{11}ClFNO_4$

molecular mass: 311.7 amu

2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propanoic acid

Figure 3: Structure and Chemical Name of CGA 185072

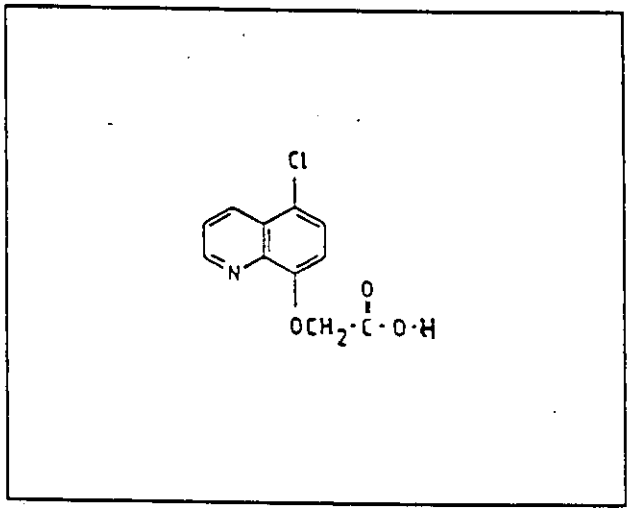


$C_{18}H_{22}ClNO_3$

molecular mass: 335.8 amu

5-Chloro-8-quinolinoxyacetic acid-1-methyl-hexyl-ester

Figure 4: Structure and Chemical Name of Metabolite CGA 153433.



$C_{11}H_8ClNO_3$

molecular mass: 237.6 amu

5-Chloro-quinolinoxyacetic acid

Figure 5: Procedure Flow Diagram