CGA 184927 (HERBICIDE)
CGA 185072 (SAFENER)

REM 138.04

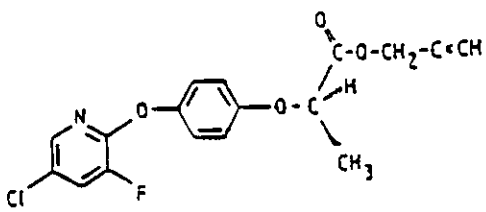
DETERMINATION OF RESIDUES OF METABOLITES CGA 193469 AND CGA 153433 BY LIQUID CHROMATOGRAPHY (HPLC)	SOIL	Apr 22, 1991 AG 2.53/A8

TABLE OF CONTENTS

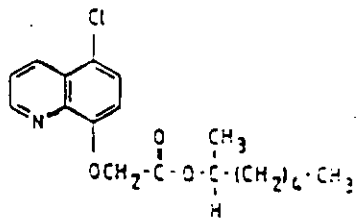
CHEMICAL STRUCTURES

PARENT COMPOUNDS

CGA 184927

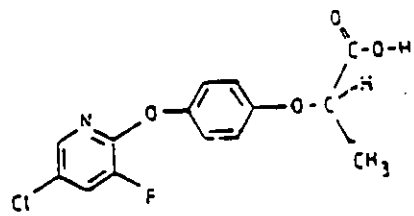


CGA 185072

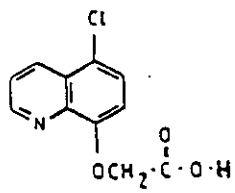


METABOLITES

CGA 193469



CGA 153433



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1. INTRODUCTION

1.1 Scope of the Method

CGA 184927 is an experimental herbicide, applied postemergence to crop and weeds. It provides excellent control of annual grasses in cereals (wheat and barley) when applied in conjunction with the safener CGA 185072.
CGA 193469 appears to be a major metabolite of the herbicide CGA 184927 in plant and soil metabolism. Likewise, CGA 153433 seems to be a major metabolite of the safener CGA 185072.
The present method permits residues of the two metabolites CGA 193469 and CGA 153433 in soil to be determined [separately] and quantitated at a lower practical level of 0.05 mg/kg.

1.2 Principle of the Method

SOIL samples are extracted with an acetone-buffer pH 3 mixture. The extracted sample must be reextracted. An aliquot of the extract is cleaned up in a solid phase extraction step on a C-18 cartridge. The analytes are separately eluted from the cartridge, CGA 153433 being in the first, CGA 193469 in the second eluate. Further purification is achieved by partitioning of CGA 193469 into hexane-diethyl ether and CGA 153433 into dichloromethane, respectively, before separate determination of the two analytes by HPLC with UV-detection under reversed phase conditions.

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 (Techne Ltd., Duxford, Cambridge, England)
2.1.2 Laboratory Shaker, Type KL-2 (E. Bühler, Tübingen, FRG)
2.1.3 Labor Centrifuge, Model "MISTRAL 2000", MSE Scientific Instruments, Crawley, West Sussex, RH10 2QQ, Great Britain
2.1.4 Vacuum manifold to accommodate solid phase extraction cartridges (build in-house)

2.2 Reagents

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

2.2.1 Acetic acid, aqueous 1 %, prepared by dilution with water (HPLC grade) from acetic acid, puriss., analytical grade, (Fluka # 45730)
2.2.2 Acetone, for residue analysis, (Merck, # 12)
2.2.3 Acetonitrile, HPLC grade, (Fluka, # 692)
2.2.4 Dichloromethane, analytical grade, (Merck, # 6050)
2.2.5 Diethyl ether, analytical grade, (Merck, # 921)
2.2.6 Ethanol, HPLC grade (Fluka, # 2855)
2.2.7 n-Hexane, for residue analysis, (Merck, 4371)
2.2.8 Hydrochloric acid, 0.1 N, prepared by dilution with water (HPLC grade) from hydrochloric acid 37 %, analytical grade (Merck, # 317)
2.2.9 Methanol, analytical grade, (Merck, # 6009)
2.2.10 Phosphoric acid, 0.1 molar, prepared by dilution with water (HPLC grade) from phosphoric acid (85 %), Merck (# 573)

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2.2.11 Water, for use in HPLC (prepared in-house)
2.2.12 C-18 Bond Elut Solid Phase Extraction (SPE) cartridge, 500 mg/3 mL, N° 607303 (Analytichem Int. Co., Harbor City, CA 90710, USA)
2.2.13 Buffer pH 3 (citrate-hydrochloric acid) made from Titrisol (Merck; # 9883), made up to 500 mL with water (HPLC grade)
2.2.14 CGA 193469 reference substance for standardisation and recoveries
2.2.15 CGA 153433 reference substance for standardisation and recoveries

2.2.16 Standard Solutions

Prepare stock solutions in acetonitrile:
CGA 193469 --> 200 µg/mL
CGA 153433 --> 100 µg/mL (due to low solubility of the compound)
For preparation of standard solutions calculate the lowest standard concentration as follows:

$$C = \frac{L \times W \times Va}{Ve \times Vf} \quad [\mu g/mL]$$

L is lower practical level [mg/kg]
W is weight of subsample [g]
Va is volume of aliquot cleaned up [mL]
Ve is volume of extract solution [mL]
Vf is final volume for determination [mL]

Example for soil: C = 0.05 x 25 x 5 / 250 x 5 = 0.005 µg/mL.

Prepare at least four standard solutions of different concentrations by diluting the stock solutions with mobile phase (section 2.4.1). Evaporate the solvent of the stock solution prior to dilution (in air stream at ~ 40 °C). Select the concentrations as required.
Typical values are: 0.005, 0.01, 0.02, 0.05, 0.10 µg/mL.

2.3 Analytical Procedure

2.3.1 Preparation of Samples and Subsamples

Prepare a representative, homogeneous soil laboratory sample from the field treated samples.
Store the laboratory sample at freezer temperature until analysis.
For analysis weigh a 25 g subsample into a 250 mL wide-mouth flask.

2.3.2 Fortification

To regularly check the performance of the method, analyze two fortified control samples with each series of analyses. To prepare these samples, add known amounts of CGA 193469 and CGA 153433 to the same uncontaminated control sample prior to extraction. Select the fortification levels to be either two- and ten times the lower practical level of determination, or in the range of the expected residues.

Prepare fortification solutions of CGA 193469 and CGA 153433 at concentrations of 10.0 µg per mL, each by appropriate dilution of the stock solutions (see section 2.2.16) with acetonitrile.
Fortify untreated soil control subsamples by adding the following amounts of the fortification solutions to each subsample, respectively:
2.5 mL, each, for 1.0 mg/kg recovery;
0.25 mL, each, for 0.1 mg/kg recovery.

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2.3.3 Extraction

Add 125 mL of acetone-buffer pH 3 (80 vol + 20 vol) mixture a soil subsample (section 2.3.1) in a wide-mouth jar. Shake for 15-30 min. at 300 cycles/min. (See section 2.8. for comment).
Filter the suspension through a Buchner funnel into a 250 mL round bottom flask, using suction. Wash filter cake and funnel with 25 mL of acetone-buffer pH 3 mixture. Transfer the filter cake back to the wide-mouth flask, add 50 mL of the acetone-buffer pH 3 mixture and shake again for 5 min. Filter again through the funnel and wash the cake with 25 mL of acetone-buffer pH 3. Combine filtrates and washings in a 250 mL volumetric flask. Adjust to the mark with acetone-buffer pH 3.
Transfer an extract aliquot of 5 mL (corresponding to 0.5 g of soil) to a 10 mL graduated test tube and remove the solvent to a small volume (~ 1 mL) in a gentle stream of air using the sample concentrator at ~ 40 °C. Make up the volume to 10 mL with buffer pH 3.

2.3.4 Cleanup by Solid Phase Extraction (SPE) on C-18 Cartridge

Condition a Bond Elut C-18 SPE cartridge consecutively with methanol, water and buffer pH 3 (3 mL, each).
Transfer the residue solution of section 2.3.3 to the C-18 cartridge. Allow it to pass dropwise through the cartridge. Discard the eluted solvent.
Elute CGA 153433 with 3 mL of water-methanol (50 vol + 50 vol) mixture. Collect the eluate in a 10 mL test tube as ELUATE I.
Evaporate the solvent in a gentle stream of air at ~ 40 °C using the sample concentrator and redissolve the residue in 5 mL of buffer pH 3 solution.
Elute CGA 193469 with 3 mL of methanol and collect this eluate in a 25 mL graduated test tube as ELUATE II.
Add buffer pH 3 (or 0.1 N hydrochloric acid) up to the 20 mL mark and continue according to section 2.3.6.

2.3.5 CGA 153433 --> Cleanup by Reextraction into Organic Solvent

Add 3 mL of dichloromethane to the ELUATE I test tube (containing CGA 153433) and shake thoroughly. Transfer the organic phase to another 10 mL graduated test tube.
Repeat the extraction procedure twice with 3 mL of the dichloromethane 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 ~ 40 °C.
Redissolve the residues in 5 mL of acetonitrile-buffer pH 3 (25vol + 75vol) for quantitation according to section 2.4.1.

2.3.6 CGA 193469 --> Cleanup by Reextraction into Organic Solvent

Add 3 mL of hexane-diethyl ether (80 vol + 20 vol) to the ELUATE II test tube (section 2.3.4; containing CGA 193469) and shake thoroughly. Transfer the supernatant organic phase to a 10 mL graduated test tube.
Repeat the extraction procedure twice with 3 mL of the hexane-diethyl ether mixture. Combine the organic phases in the test tube.
Evaporate the solvent to dryness in a gentle stream of air using the sample concentrator at ~ 40 °C.
Redissolve the residues in 5 mL of mobile phase for quantitation according to section 2.4.1.

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2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic System

2.4.1.1 For separate DETERMINATION of CGA 193469 and CGA 153433 use a HPLC single-column system with UV-detector, pump and autosampler-injector as follows or with suitable equivalents.

Detector: Kratos Spectroflow 773 UV/VIS Detector (Applied Biosystems, Ramsey, NJ 07446, USA)

Pump: Dual piston pump model 420 (KONTRON Instruments AG)

Sampling system: Automatic Sampling System MSI 660 (Kontron Instruments AG, CH-8010 Zürich, Switzerland) with Rheodyne injection valve (model 7010)

Recorder: Dual channel recorder (ABB Goerz AG, SE 120, A-1101 Wien); sensitivity set to 10 mV full scale; chart speed: 0.5 cm/min

Optional (for system automation): control and data collection unit, HP 3357 Laboratory Automation System, Hewlett-Packard, Avondale, PA 19311, USA

For determination of CGA 193469 use the following chromatographic conditions:

Column: Stainless steel, 25 cm length, 4.6 mm id., packed with Inertsil-100 C-18, particle size 10 µm (Melz, VDS GmbH, Berlin, FRG)

Mobile phase:	acetonitrile-ethanol-0.1 m phosphoric acid (20 vol + 40 vol + 40 vol)
Flow rate:	0.9 - 1.0 mL/min
Detector wave length:	226 nm
Detector sensitivity:	0.004 aufs
Retention time:	~ 9 minutes
Volume injected:	200 µL

For determination of CGA 153433 use the following chromatographic conditions:

Column: Stainless steel, 25 cm length, 4.6 mm id., packed with Inertsil-100 C-18, particle size 10 µm

Mobile phase:	acetonitrile-acetic acid 1% (25 vol + 75 vol)
Flow rate:	0.9 - 1.0 mL/min
Detector wave length:	244 nm
Detector sensitivity:	0.004 aufs
Retention time:	~ 8 minutes
Volume injected:	200 µL

2.4.2 Quantitation of Residues

Standardise the chromatographic system each time a series of samples 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 calculation of the lowest standard concentration see section 2.2.16.

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Inject 200 µL of standard solutions and final (sample) solutions. Since detector response may change on injections of coextractives, inject final and standard solutions alternatively.
Measure the response of the analyte (CGA 193469 or CGA 153433) at the characteristic retention time and calculate the reponse function and residue as detailed in section 2.9.

2.5 Interferences

Some interferences have been found to originate from reagents used (e.g. hydrochloric acid, hexane, dichloromethane etc.). In this case run "reagent blanks" through the method for locating he cause of troubles.
Different types of reversed phase analytical columns may be suitable to shift interferences in such a way as not to hinder quantitation: e.g. Nucleosil C-18; 250 x 4 mm; particle size: 7 µm (Macherey-Nagel, CH-4702 Oensingen, Switzerland).
Using alternative mobile phases such as acetonitrile-0.1 N phosphoric acid mixtures, may also result in removing interferences.
Also, applying a 2-column switch system may overcome the interference problem and save the need for further cleanup.
When running samples on a single-column system, allow for a long run time, due to eventually late eluting peaks.

2.6 Confirmatory Techniques

None developed.

2.7 Time Required for Analysis

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

2.8 Modifications and Potential Problems

Many soil types need less cleanup than described here. In this case, the SPE cleanup may be omitted.

Filtration of extracts from very finely structured soil types may be difficult or impossible. To overcome this problem, centrifugation may be applied. Due to the improved homogenicity of very fine soil, the sample size may be then be reduced to 1 - 2 grams.

In some cases 0.1 N phosphoric acid has proved to be superior to 1 % aqueous acetic acid with respect to baseline performance.

CAUTION: Due to their bipolar chemical structures, both analytes are sufficiently extracted only at a pH range near their iso-electrical point, i.e. close to pH 3. The buffer pH 3 solution is used for this reason. Soil types having a different pH-range, may alter the pH of the extract solution during extraction and lower the extraction efficiency. Thus, the extraction time of each soil type must be optimized in such a way that no alteration of the pH 3 range occures. This may mean, that a very short extraction time (e.g. 2-3 minutes) has to be observed.

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2.9 Methods of Calculation

2.9.1 Standardise the chromatographic system as outlined in sections 2.2.13 and 2.4.2.

2.9.2 Determine response (peak height or area; prefer height) of standard injections either electronically or with a ruler.

2.9.3 Plot the standard curve (amount vs. response) in a double logarithmic form or - preferably - calculate the response function:

$$\text{response} = f(\text{amount}),$$

by linear regression.

Note: In case of calculation take into account the fact, that random errors in chromatographic systems predominantly are relative to the amount, and therefore an adequate calculation procedure has to be used, e.g. linear regression by minimizing the sum of the squares of the relative deviations of all measured points from the curve.

With the amount expressed as ng, the response function will have the form:

$$\text{response} = a\,(ng) + b$$

2.9.4 From the response function calculate its inverse function, the analytical function:

$$\text{amount} = f'(\text{response})$$

or

$$ng = [(\text{response}) - b] / a$$

2.9.5 Calculate also the relative deviations from the curve of all standard points and the variance of all these deviations. Take this variance for an estimate of the variance of the chromatographic system.

2.9.6 Consider the range of definition of the analytical function to be limited by the two points (ng_{min}, $peak_{min}$) and (ng_{max}, $peak_{max}$); ng_{min} and ng_{max} being the smallest and the largest standard amount, respectively; $peak_{min}$ and $peak_{max}$ being the response calculated from the response function with ng_{min} or ng_{max}, respectively.

2.9.7 Consider $peak_{min}$ to represent the most probable estimate of the response and $peak_{min}$ minus twice the standard deviation of the chromatographic system (calculated from the variance) to be the minimum response at the 97.5% limit of confidence.

2.9.8 Divide ng_{min} by milligrams sample injected (cf. section 2.9.10) to determine the lower practical level. Make sure that the performance of the chromatographic system is sufficient to guarantee this lowest residue to be always above or equal to the statistically defined absolute limit of determination (which is about five times the instrument noise from peak to peak).

2.9.9 Measure the response of analyte - if present - at the characteristic retention time. If the response found is within the range of definition of the analytical function (cf. section 2.9.6) determine ng found by comparing the response with the plotted standard curve or by computing with the analytical function.

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2.9.10 Calculate the subsample portion injected for determination as follows:

$$\frac{W \times Va \times Vi}{Ve \times Vf} \quad [mg]$$

With the values proposed in this method (W = 25; Va = 5; Vi = 200; Ve = 250; Vf = 5) the sample portion injected is equal to 20 mg.

Calculate the amount of residue R [mg/kg] of a sample by dividing the amount N [ng] of analyte found by the subsample portion [mg] injected:

$$R\ [mg/kg] = \frac{N \times Ve \times Vf}{W \times Va \times Vi}$$

W: weight of subsample extracted [g]
N: amount analyte found [ng]
Vf: final volume for determination [mL]
Va: volume of aliquot cleaned up [mL]
Ve: volume of extract solution [mL]
Vi: volume injected for determination [µL]

2.9.11 If the response found is outside the range of definition of the analytical function do not extrapolate, but proceed as described in the following three sections.

2.9.12 If the response is above the upper limit of the range (i.e. above peak $_{max}$), repeat the final determination either with a more diluted sample solution or with more concentrated standard solutions.

2.9.13 If the response is below the lower limit of the range (i.e. below peak $_{min}$), proceed as follows:
Subtract the double standard deviation of the chromatographic system (calculated from the variance, cf. section 2.9.5) from the lower limit of the range; take 80% of this value as the lower threshold.

If the response under consideration is above this lower threshold take the difference to the lower limit for a random and/or systematic error; therefore round up ng calculated to ng $_{min}$, and report residue to correspond to the lower practical level. This is to make sure that the probability of falsely reporting a residue to be below the lower practical level (false negatives) is as low as 2.5% even if the recovery value is only 80%.

2.9.14 If the response is below the lower threshold, report the residue to be below the lower practical level.

Note: This procedure is intended to be followed for registration purposes In case of enforcement analyses the procedure has to be changed in such a way as to make sure that the chance of false positives is small.

2.9.15 Find an example how to calculate and interpret the standardisation and the quantitation of residues in Table 1.

2.9.16 Calculate recovery values from the recovery samples (cf. section 2.3.2) as

$$\% \text{ recovery} = \frac{\text{mg/kg found}}{\text{mg/kg added}} \times 100$$

Consider the analysis of the whole series to have failed if recoveries are outside the acceptable range (cf. Appendix 7.2).

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5. TABLES AND FIGURES

5.1 Table 1 EXAMPLE OF CALCULATION AND OF INTERPRETATION OF RESULTS

Note: These examples do not actually represent results generated by this method; they are general ones intended to illustrate the calculation procedure.

A. STANDARDISATION

amount (ng)	response (counts)	response calculated	relative deviation
0.5 (= ng_{min})	1199	1389 (= $peak_{min}$)	-0.137
0.5	1431	1389	0.030
1.0	2942	2580	0.140
1.0	2832	2580	0.098
2.0	5124	4961	0.033
2.0	4998	4961	0.007
6.0	12933	14487	-0.107
6.0	13647	14487	-0.058
15.0	35437	35919	-0.013
15,0 (= ng_{max})	36161	35919 (= $peak_{max}$)	0.007

y = response	response function	y = 2381 x + 199
x = amount	analytical function	x = (y - 199) / 2381
relative deviations:	average	0.000
	variance	0.0072
	standard deviation	0.085 (= 8.5%)
range of definition:	between (0.5, 1389) and (15.0, 35919)	

B. QUANTITATION OF RESIDUES

Injected portion corresponds to extract of 25 mg sample, hence lower practical level is 0.5 ng / 25 mg = 0.02 mg/kg.

- Response 27301 counts (i.e. within the range of definition): Calculation from the analytical function gives 11.4 ng, which corresponds to 0.46 mg/kg.
- Response 43411 counts (i.e. range of definition exceeded): Report residue to be >0.60 mg/kg and proceed as outlined in section 2.9.12.
- Response 968 counts (i.e. outside the range of definition): Since response is above 80% of $peak_{min}$ (1389) minus twice the standard deviation (236), i.e. above 922 counts (= lower threshold), round up residue and report 0.02 mg/kg.
- Response 817 counts (i.e. outside the range of definition): Since response is smaller than 922 counts (the lower threshold as calculated above), report residue to be <0.02 mg/kg.

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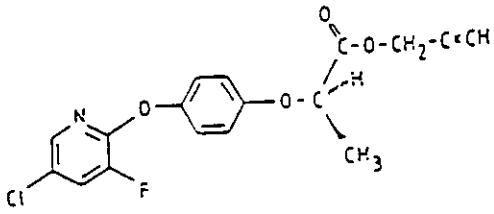
5.2 FIGURES

Figure 1: Structure and Chemical Name of CGA 184927



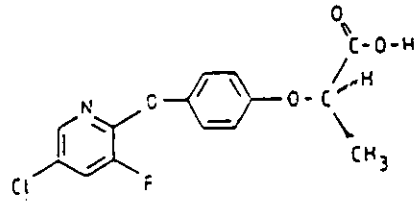
$C_{17}H_{13}ClFNO_4$

molecular mass: 349.75

IUPAC: 2-propynyl-(R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propionate

CA: 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: 311.6

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

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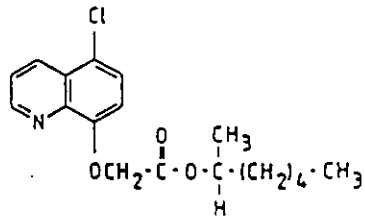
Figure 3: Structure and Chemical Name of CGA 185072

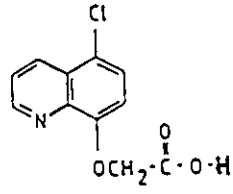


$C_{18}H_{22}ClNO_3$

molecular mass: 335.83

IUPAC/CA: 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: 237.64

IUPAC/CA: 5-chloro-8-quinolinoxyacetic acid

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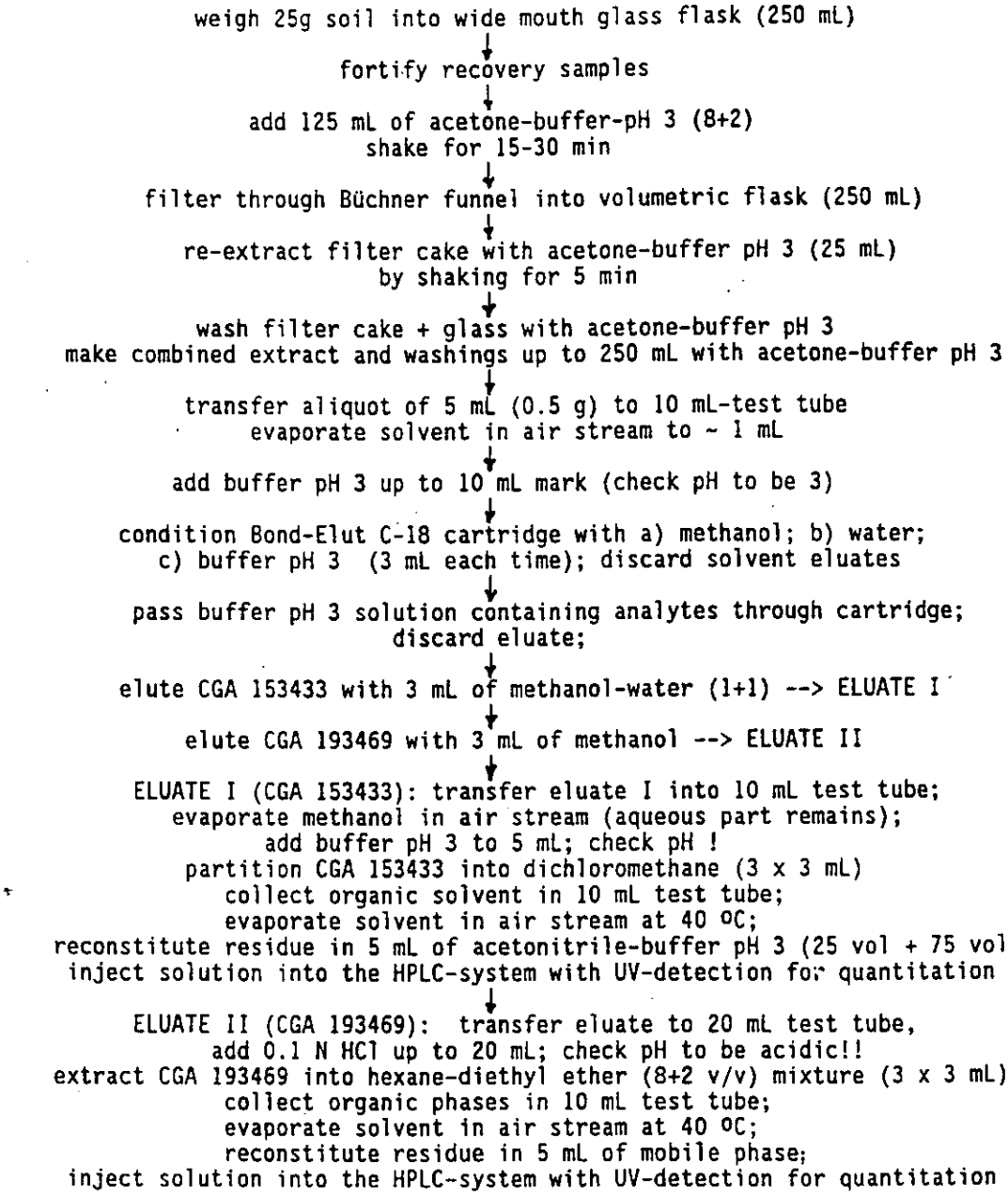
Figure 3: Procedure Flow Diagram (CGA 193469 and CGA 153433 in SOIL)



6. REFERENCES

CIBA-GEIGY AG Residue Analysis AG 2.53
Internal Residue Method REM 138.01 -> Determination of Residues of Parent Compounds (CGA 184927 and CGA 185072) in PLANT MATERIAL and SOIL

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