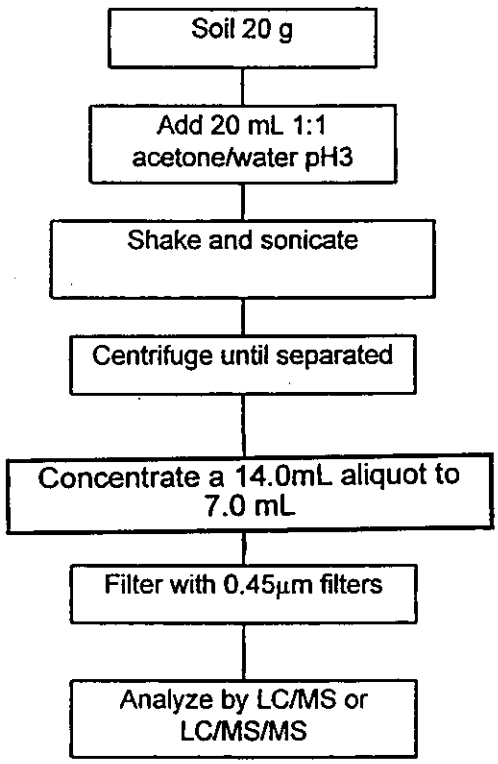
SUMMARY FLOWCHART OF ANALYTICAL METHOD



1.0 INTRODUCTION

1.1 Scope

This method sets forth the procedure for determining the residues of CGA 302371 (UE Metabolite) in soil. CGA 302371 is a metabolite of the herbicide active ingredient CGA 184927. The method is based on a in-house method developed at ETL.

1.2 Principle

An analytical method is described for the determination of residues of CGA 302371 in soil. Residues of CGA 302371 are extracted from soil using shaking and sonication with acetone/water (pH3). All residue analysis is accomplished by LC/MS or LC/MS/MS using a C_{18} column. Quantitation of results is based on a comparison of peak areas with those of known standards or by using a linearity curve. The method limit of quantitation is 0.0050 ppm.

1.3 Method Limits

The minimum limits of detection (LOD) and limits of quantitation (LOQ) for CGA 302371 in soil is 0.001 ppm and 0.005 ppm respectively.

2.0 MATERIALS

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

Acetone - glass distilled, EM Science, OmniSolv®
Acetonitrile - pesticide grade, OmniSolv®
Water, deionized - Millipore Purification System

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

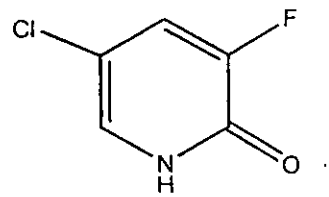
Bottles, centrifuge - polypropylene, 50 mL, Baxter
Centrifuge - Sorvall®, RC2-B with 250 mL rotor head, DuPont Instruments
Filters, 0.45μm, nylon acrodisc - Gelman
Incubator Shaker (controlled environment), New Brunswick Scientific Co.
Nitrogen evaporator with water bath - Organomation Assoc. Inc., Model No.111
Pipettes, volumetric - 10 mL
Test tubes, 50 mL and 15 mL

2.3 Solutions

Acetone:Water, pH3 (1:1), adjust the pH of deionized water to 3.0 ±0.2 with 1N HCl. Mix equal volumes of acetone and acidified water.

2.4 Analytical Standard and Chemical Structure:

CGA 302371
Chemical Name: 5-Chloro-3-fluoro-1.H. pyridin-2-one
CAS #: Not Available
Source: Novartis Crop Protection



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.

3.1.1 Weigh ~ 0.010 g (corrected for purity) of the analyte CGA 302371 into a 10 mL volumetric flask, record the exact amount, and dilute to the mark with acetonitrile. Cap and mix by inversion. The concentration of this stock standard is ~1000 µg/mL.

3.1.2 For the preparation the fortification standards of CGA 302371, 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. Concentrations of 1 and 10 ppm standards are prepared in a similar manner.

3.1.3 Prepare additional fortification and LC/MS calibration standards by serial dilution with deionized water to give standards from ~0.005 µg/mL to ~10 µg/mL.

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 within 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.

4.1.3 The analytical C18 column must resolve the analyte from any interferences that may be present, well enough to identify and quantitate CGA 302371. The gradient program may need to be modified in order to obtain this resolution.

Alternate columns may be substituted, provided they meet these criteria.

4.2 Soil Analysis
(Analysis of CGA 302371)

◆ 4.2.1 Weigh 20 g of a prepared* subsample of soil into a 250 mL polypropylene centrifuge bottle. Untreated control soils may be fortified at this point for determination of recovery. 100-200 µL of the fortification standards are spiked onto the soil and allowed to evaporate and equilbrate for ~15 min.

*Subsamples 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 20 mL of 1:1 Acetone/water, pH 3 solution to the soil, shake using a platform shaker for ~30 min.

◆ 4.2.3 Remove, shake by hand and place in a sonic bath for ~10 minutes.

4.2 Soil Analysis cont'd

4.2.4 Centrifuge the sample for at least 5 minutes at about 5000 RPM or until separated, and remove a 14.0 mL aliquot into a 16 mL test tube.

4.2.5 Using a nitrogen evaporator, evaporate off the acetone and adjust final volume to 7.0 mL with water.

4.2.6 Before transferring an aliquot to HPLC vial, filter extracts through 0.45μg filter.

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

5.0 INSTRUMENTATION - THERMOSPRAY LC/MS AND LC/MS/MS

Note: Equivalent LC/MS instrumentation utilizing other interfaces rather than thermospray interfaces can also be used. The LC/MS conditions will need to be optimized to provide adequate sensitivity, linearity and selectivity for the analysis of CGA 302371. Column switching may be required for instruments using the "heated capillary" technology. LC/MS/MS instrumentation can also be used.

5.1 Thermospray LC/MS Conditions:

Instruments used:
Finnigan (San Jose, CA) SSQ 710 with thermospray TSP-2 interface
Waters (Milford, MA) 600 MS systems controller
Waters 717 refrigerated autosampler
Column in-line: (Waters)
HPLC column: Symmetry® C18, 5 μm, 4.6 × 250 mm
(Equivalent C18 column may be used.)

Gradient Program: (linear gradient changes)

Time (min.)	% Water	% Acetonitrile
Initial	100	0
10	50	50
15	100	0

The above gradient may be modified to improve resolution and/or chromatography.

% Water - (2% acetic acid/water)
% Acetonitrile - (2% acetic acid/acetonitrile)
Flow rate - 0.9 mL/minute

5.1 Thermospray LC/MS Conditions: cont'd

Mass Calibration:

During the set-up of the thermospray LC/MS, the mass calibration is done with a 50 ppm standard of CGA 302371. Instrument should be re-calibrated if the quads are cleaned, otherwise the mass axis does not change.

Post column eluant:
Perkin Elmer HPLC/MS pump continuously adds 0.5 M aqueous ammonium acetate at a flow rate of 0.3 mL/minute.
Injection volume - 100 µL
Source temp. - 250°C
Vaporizer - 99°C
TSP on @ 9 min.
TSP off @ 15 min.
Scan time - 0.2 min.
Multiplier - 1200

Approximate Retention Times and Mass		
	m/z Target	R.T. (min.)
CGA 302371	165.1	11.45

Retention times may vary from those present above.

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

The chromatogram trace start at 11 min. since the TSP is turned off until 9 min. and equilibrates for 2 more minutes before the mass spectrometer is turned on.

5.2 Performance Criteria

First Criterion:
Run a standard solution on LC/MS corresponding to a level at 50% LOQ and obtain a signal to noise ratio of at least 9:1.

If this criteria cannot be met, optimize instrument operating parameters or change instrument method parameters such as multiplier voltage, replace the probe or clean the thermospray interface.

5.3 Performance Criteria cont'd

Second Criterion:
Run a set of standards of four or more concentration levels, from 0.005 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. The first and last sample of each run sequence must be an analytical standard.

6.0 CALCULATIONS

6.1 Response Factor (R.F.):

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

6.2 Concentration (ppm) of Analyte in Soil (wet weight basis):

$$Conc.\ (ppm) = \frac{(Peak\ Area \times Avg.\ R.F.) \times F.V.}{g.Extracted} \times A.F. \left(\frac{20}{14}\right)$$

Where: Conc.(ppm) = Concentration of analyte in µg/g(ppm)
F.V. = Final sample volume (mL)
g.Extracted = initial grams of sample extracted (wet weight)
A.F. = Aliquot factor = $\frac{\text{Extraction volume (mL)}}{\text{Aliquot}} = \frac{20\ mL}{14\ mL}$
Avg.R.F. = Average of RF bracketing a set of samples.

NOTE: Residues are calculated based on the wet weight of soil, are are not corrected for moisture content.

6.3 % Recovery:

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

Recovery Level = the recovery found in the spiked control (ppm)

Note: Although average response factors were used to quantitate CGA 302371, a calibration curve calculation would also be acceptable.