I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-152005 [N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-2-(3,3,3-trifluoropropyl)-benzene sulfonamide,, CAS #94125-34-5] and its metabolites CGA-159902 [2-(3,3,3-trifluoropropyl)-benzenesulfonamide, CAS #94125-42-5] and CGA-300406 (desmethyl CGA-152005) in water and soil. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC-MS). An ionspray atmospheric pressure ionization (API) interface is used to introduce the HPLC effluent into the mass spectrometer equipped with a single quadrupole mass analyzer. The analytes are detected by selected ion monitoring (SIM) of their unique negative ions. The structures and chemical names of the analytes are presented in Figure 1.

The limit of detection (smallest standard amount injected during the chromatographic run) is 0.125 ng for all analytes. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) is 0.05 ppb for all analytes in water and 0.5 ppb in soil.

B. Principle

A 100-ml water sample is acidified with phosphoric acid and then passed through a C18 solid phase extraction (SPE) column. CGA-152005, CGA-300406, and CGA-159902 are retained on the C18 column. The column is rinsed with appropriate solvent. The analytes are eluted from the C18 column with acetonitrile. The organic solvent in the eluate is removed to dryness using a rotary evaporator. The residue is redissolved in 50% acetonitrile/water and analyzed by LC-MS. A flow diagram for the water method is presented in Figure 2.

Soil samples (20 g) are extracted at room temperature with a mechanical shaker using 100 ml of 20% (v/v) methanol/phosphate buffer (0.05 M, pH adjusted to 8). A filtered 50-ml aliquot (ca. 10 g) of the sample extract is diluted with water, acidified, and then passed through a C18 SPE cartridge for isolation of the analytes. The remainder of the cleanup procedure is the same as for water samples. The sample is analyzed by LC-MS. A flow diagram for the soil method is presented in Figure 3.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Balance, analytical (Sartorius R160P) or equivalent.

2.0 Bottle, amber Boston round, with Polyseal-lined cap (Fisher cat. #05-563-2E) or equivalent.

3.0 Bottle, polypropylene, wide mouth (Fisher cat. #05-565-19A) or equivalent with cap (Fisher cat. #05-563-2E) or equivalent, appropriate size for soil extractions.

4.0 Centrifuge, Sorval Superspeed RC5-B (DuPont Instruments cat. #55228-9) or equivalent, with 6-place GSA rotor head (DuPont, Sorval GSA cat. #08136) or equivalent.

5.0 Cylinder, graduated, 100-ml (Fisher cat. #08-562-5C) or equivalent.

6.0 Filter paper, Whatman 2V (Fisher cat. #09-832D) or equivalent.

7.0 Flask, round bottom, 250-ml (Fisher cat. #10-067E) or equivalent.

8.0 Funnel, filter (Fisher cat. #10-326-2C) or equivalent.

9.0 Mechanical shaker, orbital (Fisher cat. #15-456-6) or equivalent.

10.0 Mixer, Vortex-Genie 2 (Fisher cat. #12-812) or equivalent.

11.0 Pasteur pipet (Fisher cat. #13-641-536) or equivalent.

12.0 pH stick, Corning (Fisher cat. #13-641-536) or equivalent pH stick or meter.

13.0 Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent.

14.0 SPE cleanup cartridge, C18 bonded phase, 1 g size (Varian Sample Preparation Products cat. #1225-6001).

15.0 Tube, concentration, 50-ml, with 19/38 ground glass joint (Fisher cat. #05-538-40B) or equivalent, with 24/19 enlarging adapter (Fisher cat. #01-035D) or equivalent.

16.0 Vials, amber, 1.5-ml (Sun Brokers, Inc. cat. #200-002) or equivalent, with teflon-lined, crimp-top seals (Sun Brokers, Inc. cat. #200-152) or equivalent.

B. Reagents and Analytical Standards

All reagents and polypropylene glycols are stored at room temperature. Solid analytical standards are stored frozen.

1.0 Acetic acid, concentrated, Optima grade (Fisher cat. #A465-250) or equivalent.

2.0 Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.

3.0 Ammonium acetate, ACS grade (Fisher cat. #A637-500) or equivalent.

4.0 Extraction solvent (soil): 20% (v/v) methanol/phosphate buffer.

5.0 Formic acid, ACS grade (Fisher cat. #A118P-500) or equivalent.

6.0 HPLC mobile phase A: 0.1% (v/v) acetic acid/acetonitrile. Add 1.0 ml of acetic acid to 999 ml of acetonitrile and thoroughly mix.

7.0 HPLC mobile phase B: 0.1/25/74.9% (v/v) acetic acid/acetonitrile/water. Combine 1.0 ml of acetic acid with 250 ml of acetonitrile and 749 ml of water. Thoroughly mix.

8.0 Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.

9.0 Phosphate buffer, 0.05 M, pH=8 ± 0.05. Dissolve 6.7 grams of sodium phosphate dibasic heptahydrate in 500 ml of purified water. Adjust the pH to 8 ± 0.05 with phosphoric acid and sodium hydroxide.

10.0 Phosphoric acid, 85% (Conc.), ACS grade (Fisher cat. #A242-1) or equivalent.

11.0 Phosphoric acid, 0.1% (v/v). Add 1.0 ml of conc. phosphoric acid to 999 ml of purified water and thoroughly mix.

12.0 Polypropylene glycol, M.W. 425 (Aldrich cat. #20,230-4).

13.0 Polypropylene glycol, M.W. 1000 (Aldrich cat. #20,232-0).

14.0 Polypropylene glycol, M.W. 2000 (Aldrich cat. #20,233-9).

15.0 PPG tuning solution. Dissolve 0.007 g PPG 425, 0.017 g PPG 1000, 0.118 g PPG 2000, and 0.021 g of ammonium acetate in 50 ml of methanol, 50 ml water, 0.1 ml acetonitrile, and 0.1 ml formic acid. Mix well. Store at room temperature in an amber bottle.

16.0 Sample diluent, 50% (v/v) acetonitrile/water.

17.0 Sodium phosphate dibasic, heptahydrate, ACS grade (Fisher cat. #S373-500) or equivalent.

18.0 Water, HPLC grade, purified in-house with a HYDRO™ purification system or equivalent.

19.0 CGA-152005, CGA-159902, and CGA-300406 analytical standards, CIBA-GEIGY Corp., P. O. Box 18300, Greensboro, NC 27419.

C. Analytical Procedure

1.0 Water

1.1 Measure a 100-ml aliquot of the water sample into a 100-ml graduated cylinder. (Note: A smaller aliquot may be used, but this will increase the limit of determination in ppb for the analyte. A larger aliquot may also be used to increase the sensitivity of the analysis.) Sample fortification, if required, should be done at this time (refer to Section II.J.2.0).

1.2 Add 1.0 ml of phosphoric acid. Shake the contents to mix.

1.3 Precondition the C18 cleanup cartridge by passing 5 ml each of methanol, acetonitrile, and finally 0.1% phosphoric acid through the cartridge. Discard the rinsate. Either positive pressure or a vacuum system may be used to aid the flow. Do not allow the packing to become dry.

1.4 Connect an appropriate reservoir on top of the C18 cartridge.

1.5 Load the aqueous sample from Step 1.2 into the reservoir and adjust the flow rate to a fast drip.

1.6 Add 10 ml of 0.1% phosphoric acid to the 100-ml graduated cylinder that previously contained the sample and shake to rinse. (Note: For soil samples, add the solution to the 250-ml round bottom flask.) Add this rinsate to the reservoir just as the reservoir becomes empty. Discard the eluate.

1.7 Rinse the cartridge with 5 ml of purified water. Discard the eluate.

1.8 Dry the cartridge under vacuum for 5 minutes.

1.9 Elute CGA-152005, CGA-300406, and CGA-159902 using 5 ml of acetonitrile. Collect the eluate in a 50-ml concentration tube.

1.10 Remove solvent using a rotary evaporator and water bath temperature of approximately 35°C until dry. Use methanol to azeotrope any remaining water.

1.11 Dissolve the residue with 50% acetonitrile/water. Mix the contents on a vortex mixer for approximately 15 seconds. Transfer the sample to an appropriate amber vial. Analyze the sample by LC-MS. (Note: Samples that are not immediately analyzed should be stored in a freezer at a temperature of <0°C.)

2.0 Soil

(Note: Soil moisture content for a representative sample for each different soil sample must be determined by appropriate methodology at the time of sample analysis.)

2.1 Weigh 20 ± 0.1 g of soil sample and place in an appropriate size centrifugable polypropylene bottle.

2.2 Sample fortification, if required for this particular sample, is done at this time (refer to Section II.J.2.0).

2.3 Add 100 ml of 20% (v/v) methanol/phosphate buffer. Place the cap on the bottle. Shake the contents vigorously for approximately 15 seconds. Place the bottle in an orbital shaker and shake the sample for approximately one hour at approximately 25°C.

2.4 Remove the sample from the shaker. Centrifuge the sample at approximately 10,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.5 Decant the extracting solvent through a Whatman 2V filter and collect 50 ml of the filtrate in a 100-ml graduated cylinder for cleanup and analysis.

2.6 Add 25 ml of purified water. Shake the contents to mix.

2.7 Transfer the contents to a 250-ml round bottom flask. Rinse the 100-ml graduated cylinder with 25 ml of water and add the rinsate to the 250-ml round bottom flask.

2.8 Remove methanol (~10 ml) from the sample with a rotary evaporator and water bath of approximately 35°C. Add 1.0 ml of conc. phosphoric acid. Mix thoroughly.

2.9 The remainder of the cleanup procedure is identical to the procedure for water. At this point refer to Step 1.3 above and follow Steps 1.3 through 1.11.

D. Instrumentation

1.0 Description and Operating Conditions - HPLC

See Table I for a description of the HPLC system and chromatographic conditions.

2.0 Description and Operating Conditions - Mass Spectrometer

CGA-152005, CGA-300406, and CGA-159902 are monitored as negative ions. A single quadrupole mass analyzer is used to separate the masses. Optimum sensitivity is achieved by selected ion monitoring (SIM) of the intense deprotonated molecular ion. See Table II for a description of the mass spectrometer instrumentation and operating conditions.

3.0 Description and Operating Conditions -Ionspray Interface

The optimized values for the ionspray interface may vary with time and may need to be periodically reoptimized by infusion of an analyte into the mass spectrometer. See Table II for a description of typical ionspray operating conditions used with the analytes in Analytical Method AG-600.

4.0 Calibration and Standardization

4.1 Calibrate and tune the mass spectrometer on a daily basis prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycol (PPG)

into the mass spectrometer using the ionspray interface while monitoring positive ions. A typical mass calibration tune with PPG is shown in Figure 4.

4.2 Detect analytes at their specific monitoring ions. Determine the specific ion to monitor for each analyte by infusing a solution of that analyte into the mass spectrometer while scanning with the quadrupole mass analyzer to find the optimum ion. Typical monitoring ions for the analytes are listed in Table III. Typical ionspray mass fragmentation spectra are presented in Figure 5.

4.3 Determine the retention time of the analytes by injecting a standard solution into the HPLC. During a series of analyses, the analyte retention time should vary no more than 2% from its mean value, on a daily basis.

4.4 Calibrate the instrument by constructing a calibration curve from detector response (chromatographic peak height or area) and the amount of analyte injected, encompassing a range from 0.125 - 1.25 ng (25-µl injections). The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Typical standard calculations are presented in Table IV. Typical standard LC-MS chromatograms are shown in Figure 6.

E. Interferences

1.0 There are no known interferences originating from the sample cleanup

procedure. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

F. Confirmatory Techniques

1.0 Although no confirmation techniques are presented here, this method provides detection based on a highly specific MS technique combined with chromatographic retention time.

G. Time Required

1.0 The sample extraction and cleanup procedure can be completed for a set of eight samples in an eight-hour working day.

2.0 Each HPLC analysis requires 20 minutes.

H. Modifications and Potential Problems

1.0 Contaminants from chemicals, solvents, glassware, and the HPLC water supply can interfere with the analysis. It is recommended that a reagent blank be run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. LC-MS is a very sensitive technique. All glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2.0 Slow degradation of the parent compound CGA-152005 is observed in acidic solutions. Degradation of other sulfonyl urea compounds has been observed in solutions with high ionic strength, even though the compound exhibited stability at that pH. Therefore, samples and standards should not be dissolved and stored in acidic or buffered solutions. Dissolved samples must be analyzed as soon as possible after dissolution as

trace acidity and salts from the cleanup extractions and from the sample matrix can create a sensitized solution, causing analyte degradation, even though the sample was dissolved in a non-sensitized solvent. Samples should be stored <0°C if they cannot be promptly analyzed after the sample extraction/cleanup procedure.

3.0 CGA-152005 is very unstable when stored in methanol. It exhibits good stability in acetonitrile and water.

4.0 CGA-152005 exhibits minor photodecomposition. Therefore, all solutions must be stored in amber vials and bottles.

5.0 CGA-152005, CGA-159902, and CGA-300406 are weakly acidic. Their retention times in reversed phase HPLC will be affected by changes in the mobile phase pH.

6.0 Analytical Method AG-600 was validated only for sandy loam and loamy sand soils from Georgia and Iowa. Other soil types or soil samples from different locations may exhibit binding or interference problems which were not observed with these samples.

7.0 Studies have indicated that CGA-300406 exhibits poor stability.

8.0 "Bumping" is sometimes observed for soil samples during the solvent removal steps via rotary evaporation. Periodic venting of the vacuum and the use of solvent traps helps minimize inadvertent losses during these steps.

I. Preparation of Standard Solutions

All standards are stored in amber bottles in a freezer (<0°C) when not in use. No analyte stability or solubility problems

have been observed in the standard solutions used in this study. (See additional comments on CGA-300406.) Sulfonyl urea compounds exhibit varying degrees of stability in aqueous; therefore it is recommended that the analytical standards in acetonitrile/water be made on a weekly basis. CGA-300406 rapidly degrades at high concentrations in acetonitrile. Therefore, the 5 ng/µl mixed solution (Step 2.0) should be prepared immediately after preparation of the 100 ng/µl stock solution. The 100 ng/µl stock solution of CGA-300406 should not be reused. A new 5 ng/µl mixed solution in acetonitrile along with subsequently prepared fortification and analytical solutions should be prepared each week.

1.0 Prepare a 100 ng/µl stock solution for each analyte by dissolving 10.0 mg of the compound in 100 ml of acetonitrile.

2.0 Prepare a 5 ng/µl mixed solution in acetonitrile by pipetting 10.0 ml of each analyte (from its 100 ng/µl stock solution in Step 1.0) into a 100-ml volumetric flask and then diluting to mark with acetonitrile. This solution is used to prepare all subsequent dilutions.

3.0 Prepare fortification standards by diluting the mixed 5 ng/µl solution (Step 2.0) with 50% acetonitrile/ water. Fortification standards should be prepared such that no more than 1 ml of the fortification solution is added to a sample. (Example: For a 100-ml water sample, the addition of 1.0 ml of a 0.50 ng/µl fortification solution will result in a fortification level of 5.0 ppb.) These solutions also serve as the analytical standards for analysis of CGA-300406, CGA-159902, and CGA-152005.

J. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Step II.C.1.11 into the HPLC. The sample solution may be diluted if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (ng) is determined by entering the value of the chromatographic peak height, or area, in the calibration response curve (Step II.D.4.4) and calculating (by computer, calculator, or manual means) the corresponding value of nanograms injected. Typical chromatograms for control and fortified control water samples are presented in Figure 7. Typical chromatograms for control and fortified control soil samples are presented in Figure 8.

2.0 Determination of Residues in Fortified Samples

Validate the method for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 0.05 ppb or more of each analyte in water and with 0.5 ppb or more of each analyte in soil.

2.1 Add an appropriate volume of a fortification solution (from Step II.I.3.0) to the sample prior to any of the cleanup steps. The total volume of the added fortification solution should not exceed 1.0 ml. For soil samples, fortify the sample and allow sufficient time for the fortification solvent to evaporate (approximately 30 min.) before proceeding.

2.2 Proceed with the sample cleanup procedure (Step II.C.1.2 for water and Step II.C.2.3 for soil).

3.0 Calculations

Calculations may be performed by computer program or manually as follows:

3.1 Calculate the analyte concentration (in ppb) for field samples from equation (1):

$$(1)\quad \text{ppb analyte} = \frac{\text{ng analyte found}}{\text{g sample injected}} \times \frac{1}{R} \times \frac{1}{P}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (4), and P is the chemical purity of the analytical standard expressed in decimal form.

The grams of sample injected for water and soil are calculated from equations (2) and (3).

$$(2)\quad \text{g sample injected (water)} = \frac{g \times V_i}{V_f}$$

where g is the grams of sample used (for water, 1.0 ml = 1.0 g), V_i is the volume (ml) of sample injected onto the HPLC column, and V_f is the final volume (ml) of the sample (from Step II.C.1.11).

$$(3)\quad \text{g sample injected (soil)} = \frac{g}{V_a + (m \times g)} \times \frac{V_a \; V_i}{V_f}$$

where,

g is the grams of soil (wet weight) used, V_a is the aliquot volume of extracted sample used for analysis (ml), V_e is the volume of extract solvent used (ml), V_i is the volume (ml) injected onto the HPLC column, m is the percent moisture in the sample, expressed in decimal form (ex. 0.1 = 10%), V_f is the final volume (ml) of the cleaned-up sample (from Step II.C.1.1I).

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (4).

$$(4)\quad R\% = \frac{\text{ppb analyte found} - \text{ppb analyte (control)}}{\text{ppb analyte added}} \times 100$$

The ppb of analyte found is calculated from equation (5).

$$(5)\quad \text{ppb analyte found} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

Residues of metabolites found in test samples may also be expressed as parent equivalents by multiplying the amount found by the ratio of the molecular weight of CGA-152005 to that of the metabolite (equation (6)).

$$(6)\quad \text{ppb CGA-152005 equiv.} = \text{ppb metabolite} \times \frac{MW\ (p)}{MW\ (m)}$$

where MW(p) is the average molecular weight of CGA-152005 (419.4) and MW(m) is the average molecular weight of the metabolite, 405.4 for CGA-300406 and 253.2 for CGA-159902.

3.2 The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the standard deviation of the determined concentration.

VIII. LIST OF TABLES AND FIGURES

TABLE I. HPLC OPERATING CONDITIONS

Instrumentation:

Perkin-Elmer Model 410 Gradient Pump
Perkin-Elmer Model ISS 200 Autosampler
FIAtron Model CH-30 Column Heater

Operating Conditions:

Column Heater: 30°C
Injection Volume: 25 µl
Mobile Phase Flow Rate: 1.0 ml/min
Column: Zorbax Rx C8, 15 cm x 4.6 mm, equipped with an Upchurch pre-column filter (0.5 µm)
Mobile Phase A: 0.1% (v/v) acetic acid/ acetonitrile
Mobile Phase B: 0.1/25/74.9% (v/v) acetic acid/acetonitrile/water
Mobile Phase Gradient Program:

Time (min.)	% A	%B
0	0	100
10	100	0
4	100	0
1	0	100
5	0	100

Total Run Time: 20 min.

Analyte Retention Times:

CGA-300406	7.8	min
CGA-159902	8.8	min
CGA-152005	10.1	min

TABLE II. MASS SPECTROMETRY OPERATING CONDITIONS

Instrumentation:

PE Sciex API 1 Single Quadrupole Mass Spectrometer
Ionspray Liquid Introduction Interface

Instrument Control and Data Collection:

MacIntosh IIfx Computer

Software:

Calibration and Mass Tuning: Tune 2.1.2
Acquisition: RAD 2.15 β
Quantitation: MacQuan 1.1.2
Display: MacSpec 3.2

All software programs written and provided by PE Sciex.

Operating Condition:

Interface Heater: 70°C
Mobile Phase Split Ratio: ~20-25:1
Curtain Gas Flow: 0.9 on gauge
Nebulizer Gas Flow: 0.9 on gauge

Typical State File Values:

Parameter	PPG Positive Ion Mass Calibration	Negative Ion Analytes
DI	Linked	100
ISV	5500	-4200
IN	650	- 650
OR	35	- 55
RO	35	- 30
M1	10	10
RE1	109	109
DM1	0..6	- 0.04
R	32	- 28
L9	- 150	100
FP	- 150	100
MU	-3600	3800

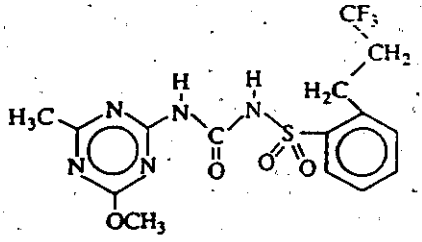
TABLE III. TYPICAL ANALYTE MONITORING IONS

Analyte	Exact Molecular Weight	Mass For Negative Ion Monitoring
CGA-152005	419.09	418.0
CGA-300406	405.07	404.0
CGA-159902	253.04	252.0

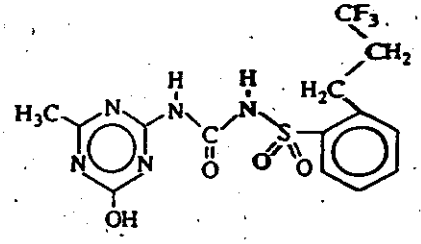
Scan Rate: 1 scan/sec

Dwell Time: 249.98 msec

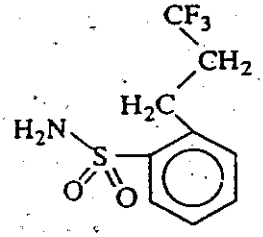
FIGURE 1. CHEMICAL NAMES AND STRUCTURES



CGA-152005
N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-2-(3,3,3-trifluoropropyl)-benzene-sulfonamide
CAS #94125-34-5
Chemical Purity: 97.1



CGA-300406
Chemical Purity: 97.8



CGA-159902
2-(3,3,3-trifluoropropyl)-benzenesulfonamide
CAS #94125-42-5
Chemical Purity: 99.6

FIGURE 2. AG-600 FLOW DIAGRAM FOR WATER

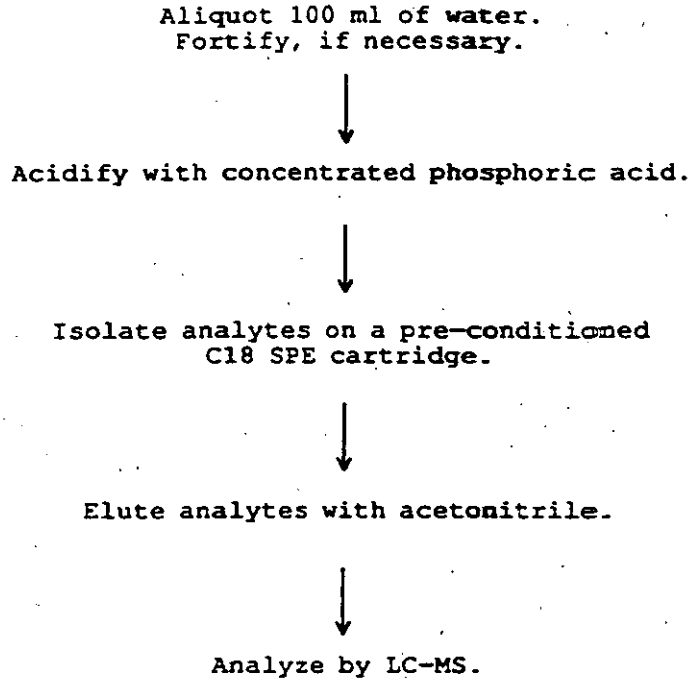


FIGURE 3. AG-600 FLOW DIAGRAM FOR SOIL