I. INTRODUCTION/SUMMARY

A. Background

Method AG-670 was originally issued May 29, 1997. However, since that time, several modifications to the original method were necessary to (1) lower the method LOQ from 10 ppb to 5 ppb and (2) account for an additional metabolite (i.e., CGA-321432). These revisions are captured in this amended method. The major points of the method remain unchanged (i.e., extraction solvent, SPE cleanup cartridge and elution solvent, mode of detection, etc.). The modifications to the method include the addition of one metabolite, increasing the soil sample size from 10 to 20 grams, using only half the sample aliquot for analysis and the substitution of dilute formic acid for water in the SPE conditioning and wash steps. These modifications have been shown to produce acceptable results using soil from North Carolina and California CGA-276854 field test site locations.

A slightly modified version of method AG-670A was developed by Ricerca, LLC (Painesville, OH) to analyze IA soil (high organic matter) with LC/MS/MS for detection in Novartis study number 190-99. These modifications are captured in Appendix 1. Once study 190-99 has been completed, method performance data and representative chromatograms will be presented in the analytical phase report.

B. Scope

This method is used for the determination of CGA-276854 (Chemical Abstracts Registry (CAS) Number: 134605-64-4, Chemical Name: Benzoic acid, 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-1,1-dimethyl-2-oxo-2-(2-propenyloxy)ethyl ester) and eight of its metabolites in soil. These metabolites are identified by the Novartis code numbers: CGA-98166, CGA-368220, CGA-368221, CGA-293730, CGA-293731, CGA-380963, CGA-321432 and CGA-350426. The structures and chemical names (as available) of the analytes are presented in Figure 1.

The compounds are separated by reversed-phase liquid chromatography and detected by mass spectrometry (LC/MS). For MS detection, a portion of the LC effluent is directed into a PE-Sciex IonSpray interface to introduce the sample into the mass spectrometer. The analytes are detected in the single quadrupole (MS) mode. For MS detection, selected ion monitoring of the negative $(M-H)^-$ or positive $(M+H)^+$ ion, depending upon the analyte, is utilized. For MS/MS analyses (not shown in this method report), characteristic

product ions are formed from passage of the parent molecular ion through the first quadrupole (Q1) into the collision cell (Q2), where fragmentation of the parent ion occurs. The fragment ions are separated in the second quadrupole (Q3) prior to detection.

The limit of detection (smallest standard amount injected during the chromatographic run) is 0.25 to 0.5 ng (depending upon instrument sensitivity) for LC/MS. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) using this LC/MS method of analysis is 5 ppb in soil.

C. Principle

Soil samples (20 g) are extracted, at room temperature, two times with 50 mL portions of 50:50 (v/v) acetonitrile:water using mechanical shaking. Samples are centrifuged and filtered after each extraction. The combined extracts are acidified with formic acid, centrifuged again if necessary, and a 50-mL aliquot placed on a rotary evaporator to remove acetonitrile. The (acidic) aqueous extracts are passed through a C18 SPE cartridge. The retained analytes are eluted from the cartridge using ethyl acetate. After rotary evaporation to remove the elution solvent, the samples are reconstituted with 25:75 v/v acetonitrile:0.1% formic acid in purified water. An aliquot of the sample is filtered (optional) into an HPLC autosampler vial for analysis by LC/MS. A flow diagram of the method is shown in Figure 2.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Balance, analytical (Sartorius R160P), or equivalent.

2.0 Bottle, 2 oz. amber glass Boston round, with TFE-lined closures (Fisher cat. #03-328-3A), or 4 oz. (Fisher cat. #03-328-3B), or equivalent.

3.0 Bottle, 250-mL polypropylene with screw cap, (Fisher cat. #05-562-23), or equivalent.

4.0 Round-bottom flask, freestanding glass, 250-mL with 24/40 tapered joint (Fisher cat. #09-552B), or equivalent.

5.0 Round-bottom flask, 100-mL with 24/40 tapered joint (Fisher cat. #10-067C), or equivalent.

6.0 Vacuum source, recirculating water aspirator (Cole-Parmer Cat. #E-07049-00), or equivalent.

7.0 Cylinders, glass graduated, 10-mL, 50-mL and 500-mL (Fisher cat. #08-552-4H, 08-552-4B and 08-552-4E), or equivalent.

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

9.0 Pasteur pipets, disposable, (Fisher cat. #13-678-7C), or equivalent.

10.0 Pipets, glass, Class A certified, assorted volumes. These pipets are used when an exact addition of liquid is required (i.e., final addition of solvent to samples).

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

12.0 Rotary evaporator traps with 24/40 tapered joints, inner vapor tube at top (Fisher cat. #K570210-0124), or equivalent.

13.0 C18 Solid phase extraction (SPE) cartridge, Varian Mega Bond Elut, 6cc, 1 gram, (Varian cat. #1225-6001).
Note: Substitutions for this item have not been evaluated.

14.0 Filter paper, medium porosity, for filtering soil extracts prior to rotary evaporation, (Fisher cat. #09-790-14E), or equivalent.

15.0 Funnels, plastic 80 mm (Fisher cat. #10-348B), or equivalent.

16.0 Centrifuge, DuPont Sorvall® Model Super T 21 with Model SL-250T rotor (4 x 250 mL), or equivalent.

17.0 Vials, clear (or amber), 11 mm, 1.5 mL (National Scientific cat. #C4011-5W) or equivalent, with Teflon-lined, snap top (pre-slit) seals (National Scientific cat. #C4011-55), or equivalent.

18.0 Optional: Syringe filters, Whatman Anotop™ 10 mm (or 25 mm), 0.2 µm (Fisher cat. #09-926-1), or equivalent.
Notes: Alternative filtration media have not been investigated.

19.0 Vacuum manifold, (Supelco cat. #5-7033), or equivalent.

20.0 SPE reservoir, plastic, 75-mL capacity (Varian cat. #1213-1012), with adapters (Varian cat. #1213-1001), or equivalent substitutions.

21.0 Pipetters, Oxford Benchmate adjustable, 200-1000 µL volume range (Fisher cat. #21-249), or equivalent. Note: These pipetters should only be used for liquid additions that do not require high accuracy or precision, e.g. acidification of the sample extract prior to rotary evaporation.

B. Reagents and Analytical Standards

All reagents and polypropylene glycols (PPG) are stored at room temperature. Solid analytical standards should be stored as indicated on the shipment documentation. The PPG mass calibration solution is stored refrigerated.

1.0 Formic acid (90%), purified (Fisher cat. #A119P-1), or equivalent.

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

3.0 Ethyl acetate, HPLC grade (Fisher cat. #E195-4), or equivalent.

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

5.0 Soil extraction solvent, 50:50 (v/v) acetonitrile:water. Combine equal volume amounts of acetonitrile and purified water and mix well. Store in glass at room temperature.

6.0 Mobile phase A. 0.4% formic acid in purified water. To one liter of purified water, add 4.0 mL of formic acid. Mix well.

7.0 Mobile phase B. 0.4% formic acid in acetonitrile. To one liter of acetonitrile, add 4.0 mL of formic acid. Mix well.

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

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

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

11.0 Ammonium formate, certified (Fisher cat. #A666-500), or equivalent.

12.0 0.5% and 0.1% formic acid. To one liter of purified water, add either 5.0 mL (0.5%) or 1.0 mL (0.1%) formic acid and mix well. Store in a glass container.

13.0 25/75 (v/v) ACN/0.1% formic acid. For each liter, combine 250 mL acetonitrile with 750 mL of 0.1% formic acid in water. Mix well and store in a glass container.

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

15.0 PPG tuning solution (for mass calibration of the LC/MS system). Dissolve 0.0014 g PPG 425, 0.0100 g PPG 1000, 0.0400 g PPG 2000, and 0.0126 g of ammonium formate in 50 mL methanol, 50 mL water and 0.1 mL of acetonitrile. Mix well. Store refrigerated in an amber glass bottle.

16.0 Test analytes tuning solutions (ca. 5 ng/µL). Add 500 µL of the 100 ng/µL single component standard solution in acetonitrile to 10 mL of 50:50:0.1 (v/v/v) acetonitrile:water:formic acid.

17.0 CGA-276854, CGA-98166, CGA-368220, CGA-368221, CGA-293730, CGA-293731, CGA-380963 CGA-321432 and CGA-350426 may be obtained from Novartis Crop Protection, P. O. Box 18300, Greensboro, NC 27419-8300.

C. Safety and Health

Whereas most of the chemicals used and analyzed for in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc., should be used).

D. Analytical Procedure

Note: All glassware, should be thoroughly cleaned and followed with a rinse of acetonitrile or methanol prior to use. The analysis system is very sensitive and false detects may occur if the glassware is not properly cleaned prior to each use.

1.0 Soil Moisture Determination

Note: You may use the soil moisture determination procedure outlined below or an appropriate site Standard Operating Procedure (SOP) for the determination of soil moisture content.

1.1 Label and record the actual weight (Wc) of a glass beaker or aluminum weighing pan of suitable size to contain the soil sample.

1.2 Add approximately 10-20 g of soil to the container, recording the weight of the container and undried soil (Wa).

1.3 Place the sample in an oven set at 100-120°C and let it dry overnight (i.e., 12-16 hours). Total drying time should not exceed 72 hours.

1.4 Take the sample out of the oven and allow it to cool to room temperature.

1.5 Record the weight of the container plus dry soil (Wb).

1.6 Calculate the moisture content using the following equation:

$$\% \text{ moisture} = \frac{W_a - W_b}{W_a - W_c} \times 100$$

where Wa is the combined weight of the container and the wet soil, Wb is the weight of the container and the dry soil, and Wc is the weight of the empty container.

2.0 Soil Extraction and Cleanup

Soil samples must be homogenized prior to analysis using appropriate sample preparation techniques.

Soil characterization data for the soil used in this validation study are presented in Table 1.

2.1 Weigh a 20 ± 0.1 gram aliquot of soil into sturdy polypropylene bottle with a screw cap. Record the sample weight. If required, sample fortification should be performed at this time (refer to Section II.K.2.0).

2.2 Add 50 mL of the soil extraction solvent (i.e., 50:50 (v/v) acetonitrile:water), replace screw cap and swirl to mix.

2.3 Place the bottle in a mechanical shaker and agitate at room temperature for approximately 30 minutes.

2.4 Centrifuge the sample for eight minutes at a speed of approximately 8000 rpm.

Note: The centrifuge settings may be adjusted as needed to obtain satisfactory results.

2.5 Decant the sample extract through filter paper into a 250-mL round bottom flask. Alternatively, vacuum filtration with a Büchner funnel and vacuum adapter may be used.

2.6 Pour a second 50-mL aliquot of the extraction solvent into the sample bottle and repeat steps 2.3 through 2.5, combining the two extracts.

Note: Shake the bottle by hand to break up the compacted soil from the first centrifugation.

2.7 Add approximately 500 µL 90% formic acid to the filtered sample extract and swirl to mix (Oxford adjustable pipetter accuracy is acceptable).

Visually inspect the sample for cloudiness or suspended solids which may indicate the need for an additional centrifugation step. If needed, transfer the sample to a 250 mL polyethylene bottle and centrifuge for approximately 5 minutes at ca. 8000 rpm. The centrifuge settings may be adjusted as needed to obtain satisfactory results.

2.8 Remove a 50 mL aliquot of the filtered sample and place on a rotary evaporator with a water bath temperature of approximately 40°C. Use a solvent trap to minimize any losses due to bumping.

Note: The step is used to remove acetonitrile from the extraction solvent and this can usually be accomplished in approximately fifteen to twenty minutes with a vacuum of 700 mm Hg. At this point the extract volume will be ca. 20 mLs (not necessary to measure).

2.9 Add approximately 25 mLs of purified water to the sample. Swirl to mix.

2.10 Condition the C18 SPE cartridge with approximately 5 mL ethyl acetate using the SPE vacuum manifold. Elute at a steady drip rate then dry using full vacuum for approximately 30 seconds.

2.11 Add ca. 5 mL acetonitrile to the cartridge and elute at a steady drip rate. Do not let cartridge go dry before adding 5-6 mL of 0.5% (or 0.1%, if acceptable results are obtained) formic acid in purified water. Elute at a steady drip rate until just a small amount of solvent remains on top of the cartridge.

2.12 Add an additional ca. 5 mL portion of 0.5% (or 0.1%, if acceptable results are obtained) formic acid in purified water, allow about half of the water to drip through, then attach a 75 mL reservoir to the cartridge.

2.13 Load the sample from step 2.9 onto the cartridge at a steady drip rate.

2.14 Without letting the cartridge go dry, add an additional 5-6 mL 0.5% (or 0.1%, if acceptable results are obtained) formic acid in purified water as a column wash.

2.15 Dry the cartridge using full vacuum for approximately 3-5 minutes.

2.16 Elute the analytes from the cartridge using ca. 10-12 mL ethyl acetate, collecting the solvent into a 100-mL round bottom flask. Rinse the neck of the flask with 5-10 mL acetonitrile. Place on a rotary evaporator with a water bath temperature of 30-40°C and evaporate to dryness.

2.17 Depending upon the method of analysis (MS vs. MS/MS) and instrument sensitivity, the reconstitution solvent volume may be varied (5.0 mL is typical). The reconstitution solvent should consist of 25% ACN and 75% of 0.1% formic acid in water. These two components may be added individually (i.e., ACN followed by aqueous) or as a premixed solution. In either case, sonicate briefly to complete dissolution.

2.18 If needed, a portion of the sample may be filtered through an Anotop syringe filter before or during transfer into an LC vial. Analyze the sample by LC/MS or LC/MS/MS. Samples which are not to be analyzed the same working day as the extraction and cleanup should be stored refrigerated at approximately 4°C. Samples that have been cold stored prior to analysis should be mixed, by inverting the sample vial 5-6 times, before loading into the autosampler tray.

2.19 Samples in which the amount found for any analyte exceeds the calibration curve must be diluted and reanalyzed. The use of class A volumetric glassware with 25:75 (v/v) acetonitrile:0.1% formic acid in purified water is suggested for these dilutions.

E. Instrumentation

1.0 Description and Operating Conditions: LC

See Table 2 for a description of the LC systems and chromatographic conditions. The optimal values for the analyte state files may vary with time and need periodic re-optimization by infusion of the analytes into the mass spectrometer. See Table 3 for a description of typical MS state file values and for conditions used with the IonSpray interface in Analytical Method AG-670A.

2.0 Description and Operating Conditions: LC/MS

Analytes CGA-98166 and CGA-276854 are monitored as characteristic positive ions while the other analytes are monitored as characteristic negative ions. See Table 4 for the LC/MS monitoring ions and acquisition parameters.

3.0 Description and Operating Conditions: LC/MS/MS

MS/MS conditions are not given in this method amendment. However, in Appendix 1, procedural modifications and instrument settings that have been used for MS/MS analysis of these analytes in soil are given. These modifications were developed by Ricerca, LLC.

4.0 Calibration and Standardization

4.1 The mass spectrometer must be calibrated and tuned on a regular basis (the frequency is at the discretion of the study director). Check the calibration and tune by infusing a standard solution of polypropylene glycols (PPG) into the mass spectrometer using the IonSpray interface while monitoring positive ions. A typical mass calibration tune with PPG is shown in Figure 3. The calibrations may also be checked by infusion of a 2-5 ng/µL solution of the test analytes dissolved in 50:50 (v/v) acetonitrile (0.1% formic acid):purified water (0.1% formic acid).

Both mass analyzing quadrupoles (Q1 and Q3) must be calibrated when operating in the MS/MS mode. An analyte calibration may be added to the PPG calibration table to ensure that the maximum ion intensity for each analyte will always be at its exact calculated mass.

4.2 Detect the analytes at their specific monitoring ions. Determine the parent ion to monitor by infusing the analyte solution into the mass spectrometer while scanning on the Q1 or Q3 mass analyzer. Determine the specific product ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic parent ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected product ion chosen to monitor will depend on the intensity of the ion fragment along with the possibility that an interference also has the same fragment ion. Typical infusion IonSpray mass spectra of the method analytes are presented in Figure 4.

4.3 At the beginning of each LC run sequence, one should inject several "warm-up" samples comprised of the analytical standards. The peak areas for these standards should be comparable to corresponding standard levels from the most recent sample set. If these values are not comparable, you may repeat the tuning procedure with infusion of the analytes to optimize the IonSpray and/or instrument state files and settings. Most importantly, these injections are used to verify that adequate sensitivity is obtained for the lowest standard level.

4.4 Determine the retention time of the analytes by injecting a standard solution into the HPLC. Should an analyte's retention time vary by more than 2% from its mean value, on a daily basis, a check of the operation of the HPLC system is recommended.

4.5 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 0.5 to 5.0 ng (100 µL injections) or 0.25 to 2.5 ng (50 µL injections). The injection volume used will depend upon instrument sensitivity. The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Standard calibrations are presented with the recovery data in Tables 5 and 6. Representative LC/MS chromatograms of analytical standards are presented in Figure 5. Chromatograms of control and fortified control soil are shown in Figures 6 and 7.

F. Interferences

1.0 Interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply. Reagent blanks should be run periodically to verify that lab contamination is not occurring.

2.0 During the course of this method validation we observed a contamination peak that closely matched the retention time and mass for CGA-98166. It was subsequently determined that the source of this contaminant was one of the lots of formic acid used in the method.

G. Confirmatory Techniques

1.0 No confirmatory method is presented. LC/MS and LC/MS/MS are considered to be highly specific techniques which combine unique MS or MS/MS data with a known chromatographic retention time.

H. Time Required

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

2.0 Samples can be analyzed overnight using an autosampler. Each LC/MS analysis requires approximately 15 minutes.

I. 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 method blank occasionally be run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. All glassware should be solvent rinsed before use to prevent contamination of control or low level samples.

2.0 No analyte stability or solubility problems have been observed when solutions have been prepared and stored as detailed in Section II.J.

3.0 Analytical Method AG-670A was validated only for the soil type listed in this method. Other soil types, or soil samples from different locations, may exhibit binding or interference problems which were not observed with these samples.

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

J. Preparation of Standard Solutions

All standards are stored in amber glass bottles when not in use. The 100 ng/µL standard solutions are stored in a freezer at ≤-10°C. The more dilute (mixed) standard solutions are stored in a refrigerator at approximately 4°C. No analyte stability or solubility problems have been observed in the standard solutions used in this study.

1.0 Prepare a 100 ng/µL stock solution for CGA-276854 and each metabolite. Weigh approximately 9 mg of the analytical standard into a (tared) 4 oz. glass amber bottle. The amount of acetonitrile to be added is calculated using the following equation:

$$V(mL) = \frac{W(mg) \times P}{C} \times 10^3$$

Where V is the volume of acetonitrile needed; W is the weight, in mg, of the solid analytical standard; P is the purity, in decimal form, of the analytical standard; C is the desired concentration of the final solution, in ng/µL; and 10^3 is a conversion factor.

For example:

To calculate the amount of solvent needed to prepare a 100 ng/µL standard solution using 9.5 mg of a 98.5% pure standard:

$$V(mL) = \frac{9.50mg \times 0.985}{100\ ng/\mu L} \times 10^3 = 93.58\ mL$$

Alternatively, you may calculate and dispense the exact amount of material needed for 100 mL of solution and prepare directly in a 100-mL volumetric flask.

Special note for CGA-98166:

This analyte is not readily soluble at a concentration of 100 ng/µL in acetonitrile. To prepare this standard solution, add 50 mL acetonitrile to the solid standard then add 5.0 mL purified water. Mix well and sonicate to dissolve. Lastly, add the remaining acetonitrile needed to obtain the final volume calculated using the equation above.

2.0 Calibration standard solutions are prepared in 25/75 (v/v) ACN/0.1% formic acid. Fortification standard solutions are prepared in acetonitrile. These solutions should be stored in amber glass bottles at ca. 4°C. The concentrations of the fortification solutions to be prepared will depend upon the desired fortification level(s). Fortification standards should be prepared so that no more than 2.0 mL of the fortification solution is added to the soil sample.

For LC/MS calibration, solutions containing 0.005, 0.01, 0.02, 0.04 and 0.05 ng/μL are suggested.

K. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Section II.D.2.18 or II.D.2.19 into the analysis system. The sample solution must be diluted if the analyte response exceeds the range of the calibration curve. Quantitation is achieved by use of the linear least squares curve fit to the calibration standards. Typical chromatograms for control and fortified control soil samples are presented in Figures 6 and 7.

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 5-ppb or more of each analyte.

2.1 Add an appropriate volume of a fortification solution (from Step II.J.2.0) to the soil sample. The total volume of the added fortification solution should not exceed 2.0 mL.

2.2 Let the sample stand for at least five minutes to allow the fortification solution to soak into the sample.

2.3 Continue with the analytical procedure.

3.0 Calculations

Calculations may be performed by computer program (preferred) or manually as shown below.

3.1 Calculate the analyte concentration (in ppb) for field incurred residues using the equation:

$$\text{RES(ppb)} = \frac{\text{Analytefound (ng)}}{\text{SWI (mg)}} \times 1000$$

where RES is the residue value in ppb, analyte found (ng) is calculated from the standard calibration curve, and SWI is the sample weight injected (mg).

The amount, in milligrams, of sample weight injected (SWI) can be calculated using the equation:

$$SWI(mg) = \frac{FW(g) \times IV(\mu L)}{FV(mL)}$$

where FW = final sample weight (g), IV = LC injection volume (µL) and FV = final volume in which sample is dissolved (mL).

The final sample weight (FW) is calculated by the equation:

$$FW(g) = \left[\frac{SWE(g) \times A1(mL)}{EV(mL) + (SWE(g) \times M(\%)/100}\right] \times \left[\frac{A2(mL)}{INV(mL)}\right]$$

where FW = final weight (g), SWE = sample weight extracted (g), A1 = aliquot 1 volume (mL), EV = total extraction solvent volume (mL), M = sample moisture in percent, A2 = aliquot 2 volume (mL), if needed, INV = interim volume (mL) is the total volume from which the 2nd aliquot is taken.

NOTE: For recovery samples, set the M% value to zero since the fortifications for these samples are based upon their wet weights. If no sample dilutions are performed, the second term in the equation (i.e., A2/INV) is equal to one.

3.2 Corrections may be made to the residue value (RES) calculated above. At the discretion of the study director, this value may be corrected to account for the average recovery and/or sample moisture.

The recovery factor, expressed as a percentage (R%), is calculated using the following equation.

$$R\% = \frac{\text{RES fortified (ppb)} - \text{RES control (ppb)}}{\text{ppb analyte added}} \times 100$$

To correct a residue value to its dry weight value, the following equation may be used:

$$SDW(ppb)=\left[\frac{CR(ppb)}{\frac{(100-M(\%))}{100}}\right]$$

where SDW = soil dry weight residue (ppb), CR = corrected soil residue, and M = soil moisture (%).

The corrected soil residue can be determined by the equation:

$$CR(ppb)=\left[\frac{RES(ppb)}{AR(\%)}\right]x100$$

where CR = recovery corrected residue (ppb), RES = residue found (ppb) and AR = average recovery (%). NOTE: Should the study director decide not to correct the residue value for recovery (i.e., use AR = 100), then CR will be equal to the RES value.

Background interferences found in the matrix blank (soil control) must be reported in the data table. In addition, an indication must be made as to whether or not these amounts were taken into account in the recovery calculations. The decision of whether to subtract any amounts found in the matrix blank from the recovery sample(s) is left to the discretion of the study director. The accuracy of the method is determined using the average recovery of the individual analytes fortified into the test substrate. The precision is estimated by the standard deviation of the determined concentration.

TABLE 2. LC SYSTEM AND OPERATING CONDITIONS

Instrumentation:

Perkin-Elmer Series 200 LC Pump and Autosampler
Perkin-Elmer Series 200 Vacuum Degasser
Eppendorf Model CH-30 Column Heater

Operating Conditions:

Column Temp.: 30°C (temperature control recommended)
Injection Volume: 50 or 100 µL (or less, as needed to obtain adequate sensitivity)
Mobile Phase Flow Rate: 1.0 mL/min
Column: Phenomenex Ultracarb 5 ODS (30), 4.6 x 100 mm, (part no. 00D-0351-EO).
Guard Cartridge: Phenomenex Security Guard Kit (part no. KJO-4282) with cartridge C18 (ODS) 4 mm L x 3.0 mm ID (part no. AJO-4287)
Mobile Phase A: 0.4% formic acid in purified water
Mobile Phase B: 0.4% formic acid in acetonitrile

Mobile Phase Gradient Program:

Step	Time, min.	%A	%B
0	3.9	70	30
1	0.1	70	30
2	1.0	30	70
3	5.0	25	75
4	2.0	25	75
5	1.0	70	30

Linear gradients used. Total LC run time of ca. 13 minutes. Slight modifications to the gradient may be required due to variations between instruments and LC columns.

Representative Analyte LC Retention Times:

Analyte	Retention time, min.	Analyte	Retention time, min.
CGA-98166	2.0	CGA-293731	4.7
CGA-368220	3.7	CGA-350426	5.0
CGA-368221	3.8	CGA-321432	5.7
CGA-380963	3.9	CGA-276854	6.6
CGA-293730	4.1		

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API I Mass Spectrometer
Sciex Liquid Introduction Interface
Instrument Control and Data Collection: Apple MacIntosh Quadra 950 Computer

Software:

Apple System 7.5
Calibration and Mass Tuning: Tune 2.5
Acquisition: RAD 2.6
Quantitation: MacQuan 1.6

All software programs written and provided by PE Sciex, except the system software by Apple. Different versions of the system and applications software may be used provided they are able to collect and process the data properly.

Operating Conditions:

Interface Heater: 70°C
Curtain Gas Flow: 1.0-1.2 L/min
IonSpray™ Gas Pressure: generally 40-50 psi (adjust to maximize analyte response)

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS (Continued)

Typical MS State File Settings

	Period 1	Period 2	Period 3	Period 4	Period 5
DI	50.00	50.00	50.00	50.00	50.00
ISV	4700.00	-3500.00	-3700.00	-3700.00	4800.00
IN	650.00	-650.00	-650.00	-650.00	650.00
OR	65.00	-59.00	-66.00	-67.00	50.00
R0	30.00	-30.00	-30.00	-30.00	30.00
M1	200.00	200.00	200.00	200.00	200.00
RE1	118.00	117.70	117.70	117.70	120.30
DM1	0.08	0.07	0.07	0.07	0.11
R1	26.00	-26.00	-26.00	-26.00	26.50
L9	-250.00	250.00	250.00	250.00	-250.00
FP	-250.00	250.00	250.00	250.00	-250.00
MU	-3800.00	3400.00	3400.00	3400.00	-3500.00
CC	10	10	10	10	10

Notes:

1.0 Typical state file values for the ppg tuning/calibration solution can be found in Figure 3.

2.0 State file values will vary slightly from instrument to instrument. These values may need slight adjustment during regular instrument optimization procedures.

TABLE 4. LC/MS MONITORING IONS AND ACQUISITION PARAMETERS

Ions Monitored:

Analyte	Exact Mass	Ion Monitored (Mode)
CGA-98166	228.03	229.0 (positive ion)
CGA-368220	309.00	308.0 (negative ion)
CGA-368221	314.07	313.0 (negative ion)
CGA-380963	350.03	349.0 (negative ion)
CGA-293730	348.01	347.0 (negative ion)
CGA-293731	434.05	433.0 (negative ion)
CGA-350426	354.10	353.0 (negative ion)
CGA-321432	435.07	434.0 (negative ion)
CGA-276854	474.08	475.0 (positive ion)

Period	Duration (min.)	Ionization Mode	Delay (min.)	Acquire (min.)	Mass(es)
1	3.00	positive	1.50	1.50	229.0
2	1.30	negative	0.00	1.30	308.0 313.0 349.0 347.0
3	0.80	negative	0.00	0.80	433.0 353.0
4	0.90	negative	0.00	0.90	434.0
5	1.00	positive	0.00	1.00	475.0

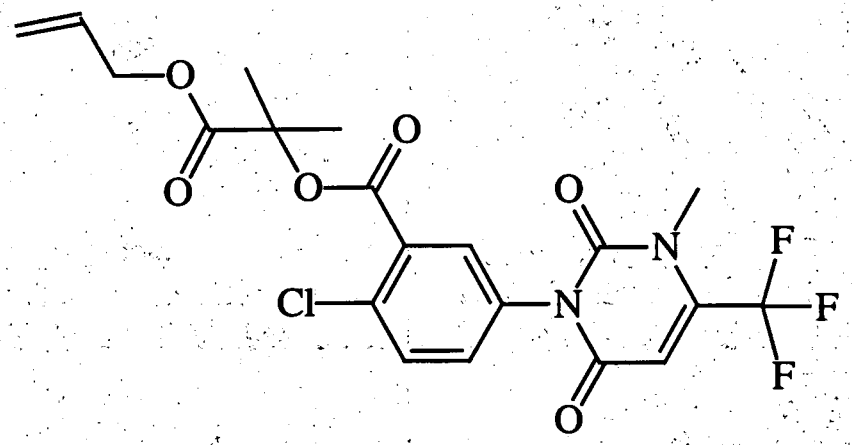
The following settings are common to all periods:

Mode: profile
Pause time: 0.02 sec.
Q1 Calibration: PPG pos. calib.
Scan rate: 1.0/sec.
Step (a.m.u.): 1.00
Scan Type: MI

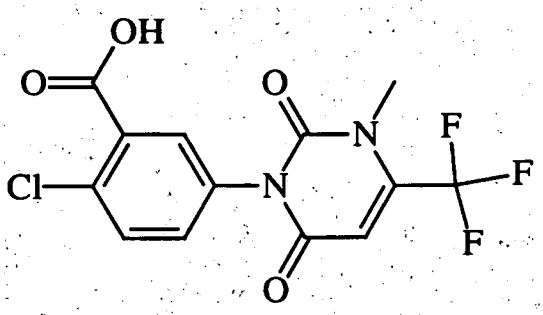
Note: Adjust period durations, as needed, to ensure that the analytes are retained within the desired period's time window.

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

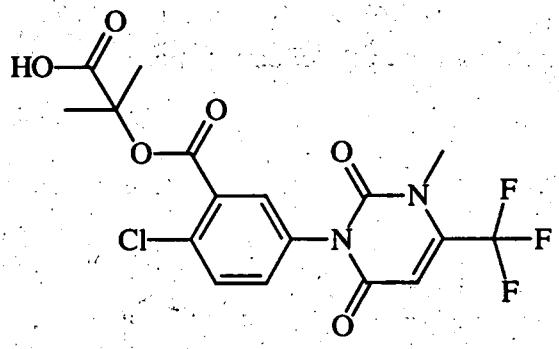
Reference Substances



Code: CGA-276854
CAS Number: 134605-64-4
CAS Name: Benzoic acid, 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-1,1-dimethyl-2-oxo-2-(2-propenyloxy)ethyl ester



Code: CGA-293730
CAS Number: 120890-58-6
CAS Name: Benzoic acid, 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]



Code: CGA-293731
CAS Number: 134605-66-6
CAS Name: Benzoic acid, 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-1-carboxy-1-methylethyl ester

FIGURE 2. AG-670A FLOW DIAGRAM

Weigh a 20 gram aliquot of soil.
(Fortify, if necessary.)

↓

Extract with 50 mL of 50:50 (v/v) acetonitrile:water
for 30 minutes using mechanical shaking
(room temperature).

↓

Centrifuge and filter extract.

↓

Repeat extraction, centrifugation and filtration step with
an additional 50 mL of extraction solvent.

↓

Acidify the filtered extract (centrifuge again if necessary to remove
suspended solids) and remove a 50 mL aliquot of sample.
Place on a rotary evaporator to remove acetonitrile.

↓

Dilute the sample with an additional 25 mL of water, load onto the
C18 SPE cartridge, then elute using ethyl acetate.

↓

Remove solvent using rotary evaporation then reconstitute the sample
with 25:75 acetonitrile:0.1% formic acid in purified water.

↓

Transfer (filter if necessary) an aliquot of the sample and
analyze by LC/MS or LC/MS/MS.