1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM070.02A is based on the extraction and cleanup procedures from Analytical Method AG-498 (Reference 1) for determination of primisulfuron-methyl (CGA136872) (Figure 1) in soil with an optional procedure for difficult samples. This method supersedes AG-498 because of LC-MS/MS technology is used as the optional determination and, as a result, the cleanup procedures are significantly simplified. The limit of quantitation (LOQ) of the method has been established at 0.01 ppm (mg/kg) for analysis of CGA136872 in soil.

This method satisfies US EPA guidelines EPA OCSPP 850.6100 (2012) and EC Guidance Documents SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).

1.2 Method Summary

Residues of CGA136872 are extracted from soil by shaking for one hour at room temperature with 90/8/2 (v/v/v) acetonitrile/H_2O/concentrated NH_4OH. An aliquot of the extract is evaporated to a small volume and diluted with 0.1 M Na_2CO_3 aqueous solution. The alkaline aqueous solution is partitioned with toluene, then the aqueous solution is acidified with diluted phosphoric acid and partitioned again with dichloromethane. The dichloromethane is evaporated and the content of the flask are dissolved in acetonitrile and the solvent evaporated again to remove any residue water. Final cleanup is performed with an Alumina Sep-Pak. The residues of CGA136872 are determined by HPLC and UV detection at 234 nm. A flow diagram for the method is presented in Appendix 1.

When difficult samples are encountered during analysis, sample extract can be diluted and directly injected on LC-MS/MS final determinations as optional procedures. A flow diagram for the optional method is presented in Appendix 2.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 3. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 4.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare an individual 100 µg/mL stock solution for CGA136872 in acetonitrile by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient CGA136872 analytical standard into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with acetonitrile and mix well to give a 100 µg/mL stock solution of CGA136872.

Alternatively, the appropriate volume of acetonitrile to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P	=	Standard purity in decimal form (P(%)/100)
V	=	Volume of methanol required
W	=	Weight, in mg, of the solid analytical standard
C	=	Desired concentration of the final solution, (µg/mL)
1000	=	Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Fortification solutions of CGA136872 should be prepared by diluting appropriate amounts of the stock solution and diluting with acetonitrile. It is recommended that the following solutions are prepared: 2.5 µg/mL and 0.25 µg/mL for fortification purposes.

2.3.3 Calibration Standards for LC-UV

Calibration standard solutions should be prepared by serial dilutions of the 100 µg/mL stock solution from Section 2.3.1. For example, transfer 1 mL of the 100 µg/mL fortification standard into a volumetric flask (50 mL) and add acetonitrile to the mark to yield a calibration standard solution at 2 µg/mL for CGA136872. Serial dilutions of the 2 µg/mL standard solution with acetonitrile will yield calibration standard solutions of lower concentrations: 0.05 µg/mL, 0.1µg/mL, 0.2 µg/mL, 0.5 µg/mL and 1 µg/mL. In general, single point calibrations are not recommended for this method (Reference 2). Calibration curves should be generated and used for quantitation of CGA136872 residues. Standards over an appropriate concentration range should be prepared as described above and a minimum of five levels of calibration standards should be used for generation of calibration curves. Typical chromatograms from LC-UV analysis of the standard solutions are shown in Figure Section.

2.3.4 Calibration Standards for LC-MS/MS

Calibration standard solutions should be prepared by serial dilutions of the 0.5 µg/mL LC-UV calibration solution from Section 2.3.3. For example, transfer 1 mL of the 0.5 µg/mL calibration standard into a volumetric flask (100 mL) and add 10/90 (v/v) acetonitrile/0.2 M ammonium acetate aqueous solution to the mark to yield a LC-MS/MS calibration standard solution at 5 ng/mL for CGA136872. Serial dilutions of the 5 ng/mL standard solution with 10/90 (v/v) acetonitrile/0.2 M ammonium acetate aqueous solution will yield calibration standard solutions of lower concentrations: 0.01 ng/mL, 0.02 ng/mL, 0.05 ng/mL, 0.1 ng/mL, 0.2 ng/mL, 0.5 ng/mL and 1 ng/mL. In general, single point calibrations are not recommended for this method (Reference 2). Calibration curves should be generated and used for quantitation of CGA136872 residues. Standards over an appropriate concentration range should be prepared as described above and a minimum of five levels of calibration standards should be used for generation of calibration curves.

Typical chromatograms from LC-MS/MS analysis of the standard solutions are shown in Figure Section.

Note: If significant matrix effects are observed for any particular matrix, matrix match standards should be used for analysis. In order to prepare matrix match standards, intermediate standard solutions should be prepared with 10/90 (v/v) acetonitrile/0.2 M ammonium acetate aqueous solution : 0.1 ng/mL, 0.2 ng/mL, 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/m, 10 ng/mL and 50 ng/mL. Matrix match standards are prepared by mixing 100 µL of the intermediate standards with 900 µL of control final extracts. For example, mix 100 µL of the 10 ng/mL intermediate standard with 900 µL of a matrix control final extract to yield 1 ng/mL matrix match standard for that particular matrix.

2.3.5 Standard Solution Storage and Expiration

Stock solution, fortification standards and calibration standards should be stored in a refrigerator when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of six months for CGA136872 is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S. G. Luxon, The Chemical Society, London (Reference 3).

Solvent and Reagent hazards

	Acetonitrile	Methanol	Phosphoric Acid	DCM	Toluene
Harmful Vapor	✓	✓	✗	✓	✓
Highly Flammable	✓	✓	✗	✓	✓
Harmful by Skin Absorption	✓	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓	✓
Causes severe burns	✗	✗	✓	✓	✓
Syngenta Hazard Category (SHC)	SHC-C, S	SHC-C, S	SHC-D,S	SHC-C, S	SHC-D,S
OES Short Term (mg/m^3)	105	310	N/A	37	37
OES Long Term (mg/m^3)	70	260	N/A	25	25

N/A not known

At present there are insufficient data available to assign a Syngenta Hazard Classification for CGA136872. It should be treated as a category SHC-D compounds until further information indicates otherwise. The Syngenta Hazard Category scale rates highly toxic chemicals as category SHC-E and non-toxic chemicals as category SHC-A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

3.1 Precautions for LC-MS/MS Determinations

a) Bottled HPLC grade ultrapure water is used to prepare the LC mobile phases, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system;

b) To prevent contamination of the instrument and to minimize possible carry-over issues, it is recommended that high level recoveries (> 0.1 ppm) and samples with expected residues greater than 0.1 ppm (mg/kg) should be diluted so that the final analyte concentration does not exceed 1 ng/mL. It may also be useful to include blank injections of 10/90 (v/v) acetonitrile/0.2 M ammonium acetate aqueous solution after injections of high level samples to clear any observed carry-over greater than 10% of the LOQ;

c) All glassware must be rinsed with ultrapure water followed by HPLC grade MeOH, acetone or acetonitrile prior to use. Polypropylene centrifuge bottles, tubes and beakers are highly recommended to be used for this method.

3.2 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.3 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), recovery samples should be prepared and included with each sample set. At least one untreated (control) and two recovery samples should be analyzed concurrently with each sample set. To each pre-weighed control soil sample, add the appropriate amount of fortification standard solution containing CGA136872 in acetonitrile (Section 2.3.2). For example, add 0.25 µg/mL fortification standard to 25 g of control soil sample to yield a 0.01 ppm recovery sample. Let the sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. The volume of fortification standard added to samples should be within the range of 0.5 - 1 mL.

3.4 Extraction

Note: A summary of the method is briefly described in flow-chart forms in Appendices 1 and 2. The procedures are normally performed with a batch of 12 samples.

a) Weigh a subsample (25 grams) from a well-homogenized, stone-free soil sample into a Nalgene bottle (250 mL). Add 125 ml of the 90/8/2 (v/v/v) acetonitrile/water/NH_4OH extraction mixture and shake for one hour at room temperature using a mechanical shaker.

b) Centrifuge for 10 minutes at 5,000 RPM using a Type *GSA* rotor.

c) Filter the sample extract through a Whatman 2V filter paper into a Boston round bottle (250 mL). Proceed to Section 3.5 for LC-UV analysis or to Section 3.7 for optional LC-MS/MS procedures if difficult samples are encountered.

3.5 Liquid-Liquid Partition

a) Measure an aliquot (25 mL) of the extract from Section 3.4 c into a graduated cylinder, pour the aliquot into a round bottom flask (100 mL) and evaporate the solvent on a rotary evaporator until acetonitrile stops distilling. (There may be 1 -2 ml of water remaining depending on the moisture content of the soil)

b) Add 50 ml of 0.1M Na_2CO_3 to the round bottom flask (100 mL), and then transfer to a separatory funnel (250 mL);

c) Partition the aqueous solution with 50 mL of toluene by shaking vigorously for 30 seconds, then, after the layers separate, drain the lower layer into another separatory funnel (250 mL). Discard the toluene phase.

d) Add 10 ml of 1.2 M H_3PO_4 to the separatory funnel containing the lower layer from Section 3.5 c and shake carefully with frequent venting until most of the CO_2 has dissipated;

e) Partition the acidified aqueous solution (pH should be 2-3) with two portions (25 mL) of dichloromethane (DCM) by shaking-vigorously each time for 30 seconds.

f) Collect both the DCM portions in a clean round bottom flask (100 mL) and evaporate on a rotary evaporator at a bath temperature of, 40-45°C. When the DCM no longer distills (there will usually be several water droplets left on the walls of the flask) stop the evaporation, immediately add 5 ml of fresh acetonitrile to the flask, swirl thoroughly and evaporate again to dryness. It is important not to leave the flask on the rotary evaporator for prolonged periods, especially before the acetonitrile evaporation (which removes any residual water) is performed.

3.6 Cleanup

a) Fit a Luer-Lok syringe (20 mL) with an Alumina-A Sep-Pak and wash the Sep-Pak first with 5 ml of 15/85 (v/v) MeOH/acetonitrile, then with 5 ml of acetonitrile. It may be necessary to start the solvent flow through the Sep-Pak by applying pressure with a pipette bulb or pressurized air;

b) Dissolve the residue in the round bottom flask (100 mL) from Section 3.5 f in 5 ml of acetonitrile and pipette into the syringe. Once the flow is started, allow the solvent to drain by gravity. When flow stops, discard the acetonitrile;

c) Elute the Sep-Pak with 15-30 mL of 15/85 (v/v) MeOH/acetonitrile, collecting the eluant in a clean round bottom flask (50 mL). Evaporate the solvent on a rotary evaporator;

d) Dissolve the contents of the round bottom flask (50 mL) in 0.5 mL of acetonitrile or some multiple of 0.5 mL for higher residue levels;

e) Pipette the sample into an injection vial for HPLC-UV analysis (Section 4.0).

3.7 Optional Procedures

a) Filter 5 mL of the sample (Section 3.4 c) through a 0.45 µm syringe filter, and then transfer an aliquot (0.5 mL) of the filtered sample into a polypropylene centrifuge tube.

b) Dilute the sample to the 10 mL mark with 90/10 (v/v) 0.2 M ammonium acetate aqueous solution/ acetonitrile. Cap and vortex well to yield the final fraction. Transfer ~1 mL of the final fraction into an injection vial for LC-MS/MS analysis (Section 5.0).

3.8 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples. One skilled analyst can complete the analysis of 20 samples in 1 day (8 hour working period).

3.9 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

4.0 FINAL HPLC-UV DETERMINATION

Instrument:	Perkin-Elmer Series 4 Liquid Chromatograph with an LC8SB Variable Wavelength UV Detector, an ISS-100 Sampling System, and a Chromatographics 3 Data Handling System or an equivalent HPLC pump and UV detector with or without automated data acquisition
Column:	Zorbax-ODS, 4.6 x- 250 mm or Phenomenex Luna C18, 250 mm, 4.6 mm, 5 µm
Mobile Phase:	Acetonitrile/0.02 M KH_2PO_4/0.02 M H_3PO_4 (65/28/7, v/v/v) or Acetonitrile/0.02 M KH_2PO_4/0.02 M H_3PO_4 (60.5/31.6/7.9, v/v/v) for the alternative Luna HPLC column

Flow Rate:	1.0 ml/min, Isocratic
Temperature:	Ambient
Attenuation:	4
Detection:	Variable Wavelength UV Detector set at 234 nm
LOD:	1.0 ng
Injection Volume;	20 µL
Chart Speed:	1.0 cm/min. from 4.5 to 10 minutes; 0.5 cm/min. all other times
Retention Time:	5.2 minutes for Zorbax column or 8.8 minutes for Luna column*

*The range of retention times is due to variations in ambient temperature.

The following instrumentation and liquid chromatographic conditions are suitable for analysis of CGA136872. Other instrumentation can also be used, though optimization may be required to achieve the desired separation and mass spectrometer sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

5.0 FINAL HPLC-MS/MS DETERMINATION (OPTIONAL)

The following instrumentation and liquid chromatographic conditions are suitable for analysis of CGA136872. Other instrumentation can also be used, though optimization may be required to achieve the desired separation and mass spectrometer sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

5.1 LC-MS/MS Instrumentation Parameters

HPLC System	Agilent 6490 LC system (H Class) with Sample Manager
Detector	Agilent 6490 Triple Quadrupole mass spectrometer
Flow Rate:	0.3 mL/min
Column:	Zorbax Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm
Column temperature:	40 °C
Injection Volume:	10.0 µL
Run Time:	9 minutes
Retention Times:	4.5 minutes under the following isocratic chromatographic conditions
Mobile Phase A:	97% 0.2 M ammonium acetate in HPLC water
Mobile Phase B:	3% acetonitrile

Mass Spectrometer Conditions

Interface:	XESI
Curtain gas:	14 L/min
Temperature:	150 °C
Capillary (V):	3000
V Charging:	1500
Nebulizer (psi):	45
Sheath gas heater:	300
Sheath gas flow:	12

MRM Conditions	Primisulfuron-Methyl
MS1:	469.1
MS2:	253.9
MS1 Resolution:	Wide
MS2 Resolution:	Wide
Dwell time:	100
Frag (V):	380
Collision Energy (V):	0
Cell Acc (V):	7
Polarity:	Positive

5.2 Data Acquisition

Peak integration and peak area count quantitation can be performed by ChemStation data handling software for HPLC analysis and MassHunter for LC-MS/MS analysis. A best-fit, linear regression equation is derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte.

Typical LC-MS/MS chromatograms from analysis of standard solutions and soil samples are shown in Figure Section.

5.3 Confirmatory Procedures for CGA136872

Final determination by LC-MS/MS with the transition for CGA136872 is considered to be highly specific; hence no further confirmatory conditions are included.

6.0 CALCULATION OF RESULTS

6.1 Multi-Point Calibration Procedure

The weight/weight concentration of CGA136872 residues in an unknown sample may be calculated in ppm (mg/kg) as follows:

a) Prepare calibration standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10 x LOQ). At least five levels of concentrations within this range should be prepared;

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to CGA136872. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions;

c) Generate calibration curve and parameters using an appropriate regression package;

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard injected on column (pg), m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Calculate the weight/weight concentration in the sample, expressed as ppb (µg/kg), as follows:

$$\text{Analyte (ppm)} = \frac{\text{Analyte Injected on Column (pg)}}{\text{Matrix Injected on Column (mg)}}$$

Where analyte injected on column (pg) is calculated from the standard calibration curve and sample matrix injected on column is calculated as follows:

$$\text{Matrix Injected (mg)} = \left(\frac{\text{Sample Wt (g)}}{\text{Extract Vol * (mL)}}\right) \times \left(\frac{\text{Aliquot Vol (mL) x Injection Vol (µµL}}{\text{Final Volume (mL)}}\right)$$

$$\text{Extract Volume*} = \text{Extraction Solvent Volume (mL)} + \text{Sample Weight (g)} \times \text{Moisture(\%)}$$

Note: The moisture (%) can be determined experimentally from water content determination of the matrix.

f) Determine the recovery factor by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery factor as a percentage (R%) by the equation:

$$\text{Recovery Factor} = \frac{\text{ppm Found in Recovery Sample - ppm Found in Control}}{\text{ppm Fortified}}$$

g) If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Analyte (ppm)} = \frac{\text{Analyte (ppm)} \times 100}{\text{Average Percentage Recovery}}$$

6.2 Single Point Calibration Procedure

CGA136872 may be calculated in ppm (mg/kg) for each sample using a mean standard response from each of the injections bracketing the sample as follows:

a) Make repeated injections of a standard containing CGA136872 at an appropriate concentration into the LC-UV or LC-MS/MS operated under conditions as described in Sections 4 and 5. When a consistent response is obtained, measure the peak area obtained for CGA136872.

b) Make an injection of each sample solution and measure the area of the peak corresponding to CGA136872.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

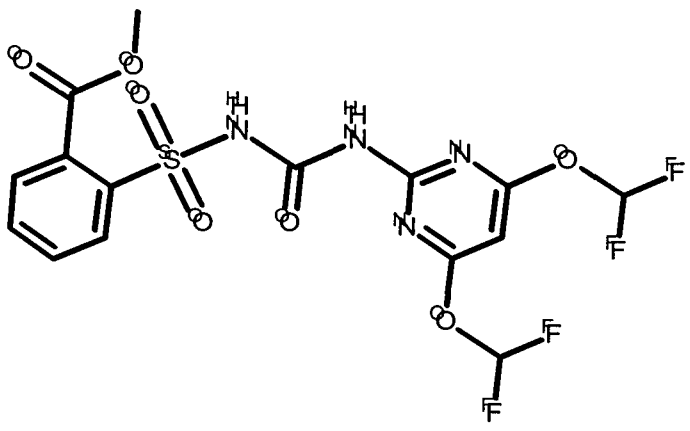
d) Calculate CGA136872 in the sample, expressed as ppm (mg/kg) using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue (ppm)} = \frac{\text{Peak area (SA)}}{\text{Peak area (STD)}} \times \frac{\text{Standard amount (pg) injected on clumn}}{\text{Matrix injected (mg) on column}}$$

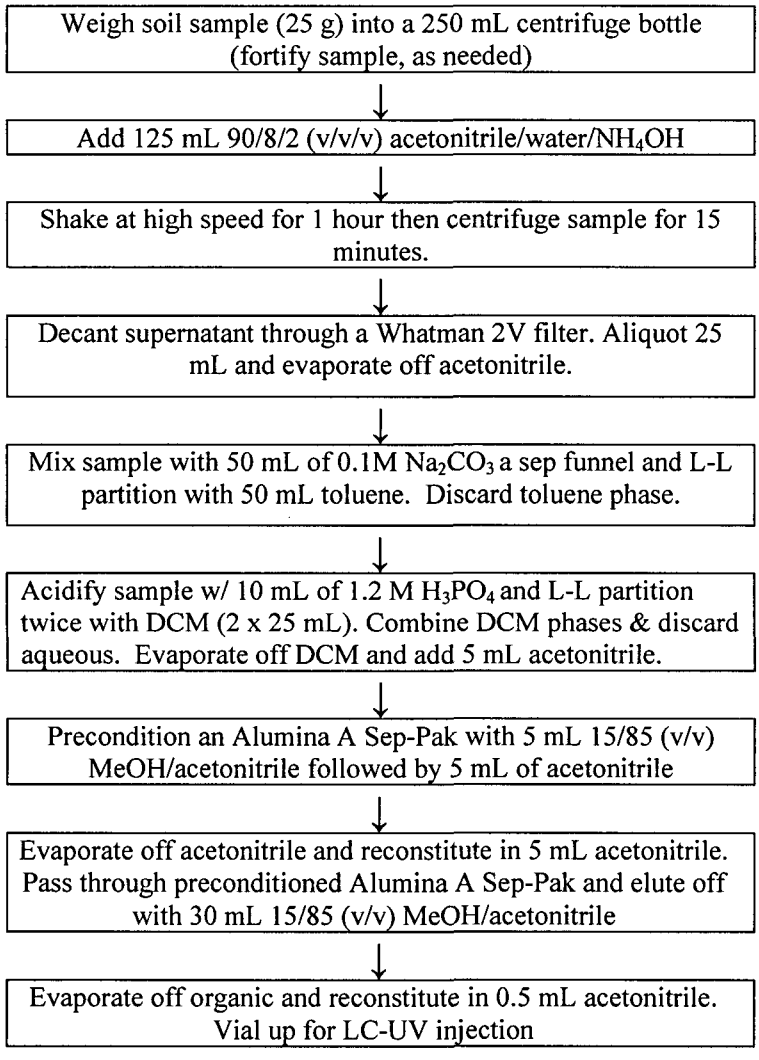
Peak area (SA) = Peak area response for unknown sample
Peak area (STD) = Average peak response for bracketing standards

Note: Although single point calibration may be used for quantitation it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 2).

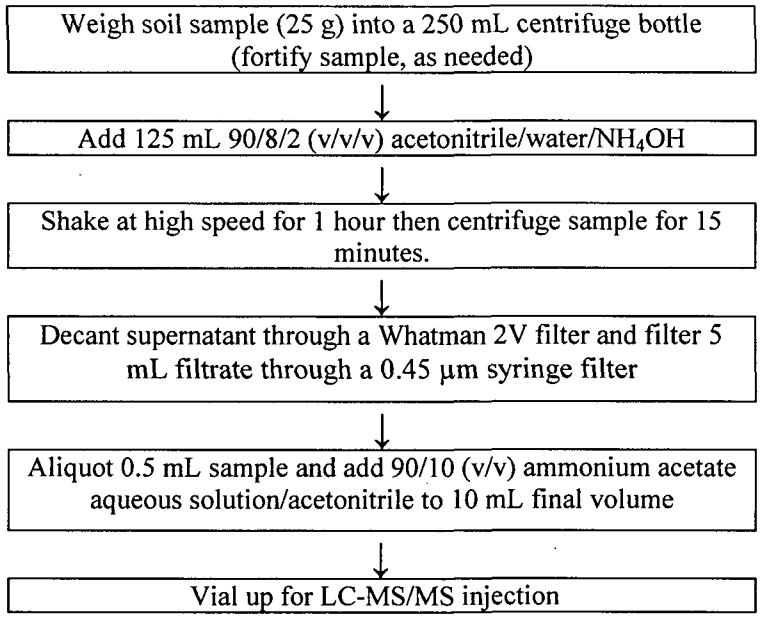
FIGURE 1 Chemical Structure

Compound	
Common Name:	Primisulfuron-Methyl
Code Name:	CGA136872
IUPAC Name:	2-{3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl} benzoic acid methyl ester
CAS Number:	86209-51-0
Molecular Formula:	$C_{15}H_{12}F_4N_4O_7S$
Molecular Weight:	468.3

APPENDIX 1 Method Flow-Chart



APPENDIX 2 Optional Method Flow-Chart



APPENDIX 3 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General lab glassware	General lab glassware	www.thermofisherscientific.com
General lab plastic-ware	General lab plastic-ware	www.thermofisherscientific.com
Autosampler vials	Snap cap, 2 mL size	www.thermofisherscientific.com
Filter paper, Whatman 2V	24 cm	www.thermofisherscientific.com
Mechanical shaker (Eberbach) or equivalent		www.thermofisherscientific.com
Rotary evaporator (Bachi) or equivalent		www.thermofisherscientific.com
Sep-Pak, Alumina-A		www.waters.com
Syringe, Luer-Lok	20 mL	www.thermofisherscientific.com

APPENDIX 4 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Ultrapure water	Optima Grade	www.thermofisherscientific.com
MeOH	Optima Grade	www.thermofisherscientific.com
Acetonitrile	HPLC Grade	www.thermofisherscientific.com
Ammonium acetate	ACS Reagent Grade	www.thermofisherscientific.com
Ammonium hydroxide, Conc.	ACS Reagent Grade	www.thermofisherscientific.com
Dichloromethane, (DCM)	HPLC Grade	www.thermofisherscientific.com
Toluene	HPLC Grade	www.thermofisherscientific.com
Potassium dihydrogen phosphate	ACS Reagent Grade	www.thermofisherscientific.com
Phosphoric acid (85%)	ACS Reagent Grade	www.thermofisherscientific.com
Sodium carbonate	ACS Reagent Grade	www.thermofisherscientific.com
CGA136872 analytical standards	GLP Certified	Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

a) 15/85 (v/v) MeOH/acetonitrile, prepared by mixing 150 mL of MeOH with 850 mL of acetonitrile;

b) 0.2 M ammonium acetate aqueous solution, prepared by dissolving 15.4 grams of ammonium acetate in 1000 mL of ultrapure water;

c) 0.1 M sodium carbonate, prepared by dissolving 10.6 grams of sodium carbonate in 1000 mL of ultrapure water;

d) 1.2 M phosphoric acid, prepared by mixing 100 mL of 85% phosphoric acid with 600 mL of ultrapure water;

e) 90/8/2 (v/v/v) acetonitrile/water/ammonium hydroxide, prepared by mixing 900 mL of acetonitrile with 80 mL of ultrapure water and 2 mL of concentrated ammonium hydroxide;

f) 0.02 M phosphoric acid, prepared by mixing 10 mL of 1.2 M phosphoric acid with 590 mL of ultrapure water;

g) 0.02 M potassium dihydrogen phosphate, prepared by mixing 2.7 grams of potassium dihydrogen phosphate in 1000 mL of ultrapure water;