2.0 INTRODUCTION

This final report describes the independent laboratory validation (ILV) of Syngenta Analytical Method CIGPSM1 "Primisulfuron-Methyl – Independent Laboratory Validation of Residue Method (CIGPSM1) for the Determination of Primisulfuron-Methyl (CGA136872), CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Water by LC-MS/MS" (Reference 1) as performed by ADPEN Laboratories, Inc. (ADPEN). The analytical method is presented in Appendix 2 and the modified procedure is presented in Appendix 3.

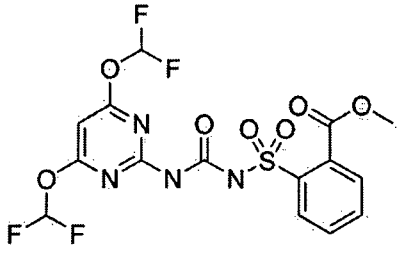
This study was designed to satisfy harmonized guideline requirements described in EPA Guideline OCSPP 850.6100 (2012) (Reference 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (Reference 3).

3.0 MATERIALS AND METHODS

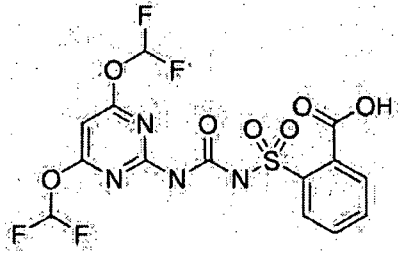
3.1 Test/Reference Substance

The test/reference substances were obtained from Syngenta Crop Protection, LLC. The following test/reference substances were used:

Compound Code Number:	**CGA136872**
CAS Number:	86209-51-0
IUPAC Name:	2-{3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl)-ureidosulfonyl}benzoic acid methyl ester
Batch Identification:	AMS 256/2
Molecular Formula:	$C_{15}H_{12}F_4N_4O_7S$
Molecular Weight:	468.3
Purity:	99.5%
Expiration Date:	End of April 2015
Storage Conditions:	< 30 °C

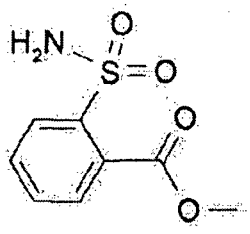


Compound Code Number:	**CGA191429**
Batch Identification:	GAN-XXIV-48-1
Molecular Formula	$C_{14}H_{10}F_4N_4O_7S$
Purity:	96.8%
Expiration Date:	August 31, 2015
Storage Conditions:	Refrigerator



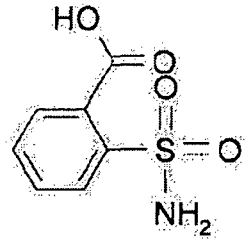
Compound Code Number: **CGA120844**

Batch Identification:	JAK-11-21
Molecular Formula	$C_8H_{11}N_1O_4S$
Purity:	99.8%
Expiration Date:	August 31, 2015
Storage Conditions:	Refrigerator



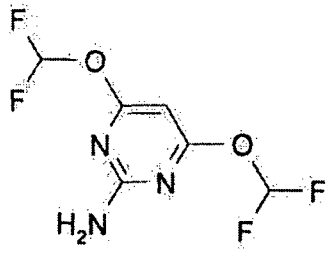
Compound Code Number: **CGA177288**

Batch Identification:	MCO-VI-32-2
Molecular Formula	$C_7H_9N_1O_4S$
Purity:	98.0%
Expiration Date:	August 31, 2015
Storage Conditions:	Refrigerator



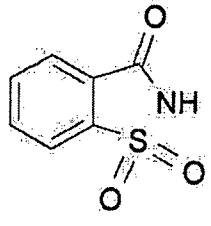
Compound Code Number: **CGA171683**

Batch Identification:	WFH-1-15
Molecular Formula	$C_6H_6F_4N_3O_2$
Purity:	97.0%
Expiration Date:	August 31, 2015
Storage Conditions:	Refrigerator



Compound Code Number: **CGA27913**

Batch Identification:	DAH-XVIII-81
Molecular Formula	$C_6H_7N_1O_3S$
Purity:	99.8%
Expiration Date:	August 31, 2015
Storage Conditions:	Refrigerator



The test/reference substance (analytical standard) used in this study was procured from the Sponsor and stored as directed. Characterization data for the test/reference standard are maintained by the Sponsor, Syngenta Crop Protection, LLC. The Certificates of Analysis are included in Appendix 4.

3.2 Test System

The test system evaluated in this study was ground water. The control sample used in this study was characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0048240. GLP characterization results are presented in Appendix 5 and summarized below.

Sample ID	Water Type	pH	Calcium (ppm)	Magnesium (ppm)
RIMV00312-0002	Ground Water	7.5	16	4.5

A control water sample was sent from Syngenta to ADPEN on August 19, 2013 and received on August 20, 2013. Upon receipt, the sample was logged in and stored in freezer E-23, which had temperature ranges during the course of this study of −29 to −6.5 °C. Prior to analysis, the sample was sub-sampled and unique laboratory codes were assigned to each sub-sample and are cross-referenced on each page of the detailed residue reports to the Syngenta sample number. Sample extracts were stored in refrigerator E-57 and E-20 while awaiting LC-MS/MS analysis. The average temperature during the course of this study for this refrigerator was 5 ± 2 °C.

The control sample was checked for contamination prior to use in this ILV study by employing the same extraction and detection method as described in Syngenta Method CIGPSM1.

3.3 Apparatus

The equipment and apparatus used for the method validation were as outlined in the method. Identical or equivalent equipment was used, as permitted by the method.

3.4 Reagents

Reagent	Description	Supplier
Acetone	HPLC grade	EMD
Acetonitrile	HPLC grade	EMD
Ethyl Acetate	HPLC grade	EMD
Methylene chloride (CH_2Cl_2)	HPLC grade	EMD
Water	HPLC grade	EMD
Acetic acid	Analytical Reagent	EMD
Ammonium acetate	Analytical Reagent	Sigma Aldrich
Phosphoric acid, 85% (conc.)	Analytical Reagent	Fisher Scientific
Sodium sulphate anhydrous	Analytical Reagent	Fisher Scientific
CGA136872 analytical standard	GLP certified	Syngenta
CGA191429 analytical standard	GLP certified	Syngenta
CGA120844 analytical standard	GLP certified	Syngenta
CGA177288 analytical standard	GLP certified	Syngenta
CGA171683 analytical standard	GLP certified	Syngenta
CGA27913 analytical standard	GLP certified	Syngenta

3.4.1 Preparation of Reagents

Reagents were prepared as described in the method.

3.4.2 Preparation of Stock Standard Solutions

Approximately 25 mg of each analytical standard was weighed into a 25-mL volumetric flask. The volume was brought up to the mark to prepare a 1 mg/mL stock solution of CGA136872, CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913. Stock solutions were stored in freezer E-51.

3.4.3 Preparation of Fortification Standard Solutions

Untreated control water samples were fortified using 0.1 mL of the appropriate fortification standard at LOQ (0.05 ppb) and 10×LOQ (0.5 ppb) concentrations as per the method. Fortifications used in this method validation are as follows:

Matrix	Standard Concentration (ng/µL)	Aliquot Volume (mL)	Dilution Volume (mL)	Final Concentration (ng/µL)
Water	10	0.5	50	0.1
	0.1	5.0	50	0.01

3.4.4 Preparation of Calibration Standard Solutions

Mixed-standard calibration solutions were prepared by volumetrically diluting intermediate standard solutions with 0.1% acetic acid. The following table is a summary of the actual preparation of calibration solutions:

Parent Concentration (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)	Final Concentration (ng/µL)
10	0.5	50	0.1
0.1	2.0	20	0.01
0.1	1.0	20	0.005
0.1	0.5	20	0.0025
0.1	0.5	50	0.001
0.01	1.0	20	0.0005
0.01	0.5	20	0.00025
0.01	0.5	50	0.0001
0.001	1.0	20	0.00005
0.001	0.5	20	0.000025
0.001	0.5	50	0.00001

4.0 ANALYTICAL PROCEDURE

Each validation set included a reagent blank, two control water samples, five control water samples fortified at LOQ, and five control samples fortified at 10× LOQ.

Syngenta Analytical Method CIGPSM1 was modified for the second trial and is described below. A summary of the modified procedure is presented in a flowchart in Appendix 1. Analytical method CIGPSM1 is presented in Appendix 2 and the modified procedure is presented in Appendix 3.

4.1 Extraction

1. A 20-mL aliquot of the control water sample was measured into a 50-mL polypropylene centrifuge tube.
2. Recovery samples were fortified with known amount of mix fortification standard solutions.
3. Concentrated acetic acid (20 µL) was added to the sample and mixed well to acidify the sample.

4.2 Final Fraction

1. The centrifuge tube was stoppered and the sample was shaken vigorously for 10 seconds to yield sample final fraction.
2. An aliquot (approximately 1.5 mL) was transferred from the sample to an autosampler vial.
3. Final determination as done by LC-MS/MS.

4.3 Modifications

The following modifications were made to the original procedure:

1. Step 1.1 was modified to use a smaller sample aliquot (20 mL instead of 100 mL).
2. Step 1.2 was modified to use 20 µL of acetic acid instead of 2.0 mL of phosphoric acid to acidify the sample.
3. Steps 1.3 through 1.15 were bypassed.
4. Step 1.16, filtration through a 0.45 micron filter, was not used during this validation study, but may be necessary if particulates are visible.
5. The concentration steps in 1.18 and 1.20 were not necessary.
6. Final determination, steps 1.19 and 1.21, was conducted under one set of instrument conditions rather than two.

4.4 Instrumentation/Operating Conditions

4.4.1 Chromatographic Conditions

HPLC Instrument:	Agilent 1200 SL			
Column:	Atlantis® T3 150 mm × 3.0 mm, 3 µm			
Column temperature:	40°C			
Injection volume:	20 µL			
Flow rate:	500 µL/min			
Mobile phase A:	0.1 mM Ammonium Acetate in HPLC Water			
Mobile phase B:	Methanol			
Gradient Step Table:	Step	Time (min)	A (%)	B (%)
	0	0	97	3
	1	5.2	45	55
	2	5.6	0	100
	3	8.5	0	100
	4	8.6	97	3
	5	11	97	3

4.4.2 Mass Spectrometer Conditions

Mass Spectrometer:	Agilent 6490 Series QQQ
Ion Mode:	ESI+Agilent Jet Stream
Polarity:	Negative
Gas Temp (°C):	150 °C
Gas Flow (L/min):	14
Nebulizer (psi):	45
Sheath Gas Heater:	300
Sheath Gas Flow:	12
Capillary (V):	3000
V Charging:	1500
Scan type:	MRM

MRM Conditions	Q1 *m/z*	Q3 *m/z*	Retention Time (min)	Dwell time	Frag (V)	CE (V)	Cell Ace (V)
Quantification Ions							
CGA136872	466.99	226	7.2	10	380	12	7
CGA191429	453.01	175.9	6.4	200	380	4	7
CGA120844	214.02	181.9	4.4	200	380	4	7
CGA177288	199.99	155.7	3.0	200	380	8	7
CGA171683	226.02	175.9	7.5	200	380	8	7
CGA27913	181.89	105.9	2.1	500	380	20	7
Confirmation Ions							
CGA136872	466.99	175.9	7.2	10	380	32	7
CGA191429	453.01	155.9	6.4	200	380	28	7
CGA120844	214.02	41.8	4.4	200	380	36	7
CGA177288	199.99	92	3.0	200	380	20	7
CGA171683	226.02	125.9	7.5	200	380	20	7

APPENDIX 1 Modified Method Flow Chart

Measure 20 ml of water sample into a 50 ml polypropylene centrifuge tube.

↓

Fortify recovery sample, if needed

↓

Add 20 µl of concentrated acetic acid to the sample

↓

Mix well to acidify the sample

↓

Stopper the polypropylene centrifuge tube

↓

Shake the sample vigorously for 10 seconds to yield final fraction

↓

Transfer an aliquot from the final fraction to amber vial

↓

Sample is ready for LC/MS/MS analysis

↓

If there are visible particles, filter the sample with 0.45 µm Nylene® filter

Method No.: CIGPSM1

Subject: **Determination of Primisulfuron-Methyl (CGA-136872) and its Metabolites in Water and Soil by High Performance Liquid Chromatography Thermospray Mass Spectrometry (TSP-LC/MS)**

Revision No.: 2 **Effective Date: 6/16/91** **Supersedes: Rev. 1 (Draft)**

I. INTRODUCTION/SUMMARY

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A. Scope

This method is used for the determination of primisulfuron-methyl (CGA-136872, 3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl)-1-[2-methoxycarbonylphenyl sulfonyl] urea) and its metabolites CGA-191429 (2-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]aminocarbonyl-aminosulfonylbenzoic acid), CGA-120844 (methyl 2-[aminosulfonyl]benzoate), CGA-177288 (2-[aminosulfonyl]benzoic acid), CGA-171683 (4,6-bis[difluoromethoxy]-2-aminopyrimidine), and CGA-27913 (1,2-benzisothiazol-3(2H)-one,1,1-dioxide) in water and soil with high performance liquid chromatography/mass spectrometry (LC/MS). A thermospray interface is used to introduce the HPLC column effluent into a triple stage quadrupole mass spectrometer, (TSP-LC/MS/MS). The structures and chemical names of the compounds are presented in Figure 1. Five analytes are analyzed by selected ion monitoring in one run by either negative ion MS, negative ion MS/MS, or positive ion MS/MS. The sixth analyte (CGA-177288), is analyzed by negative ion MS/MS using a different analytical column and mobile phase composition. The approximate instrument detection limits and detection method used for each compound are summarized in Tables III and IV. Reporting limits of 0.05 ppb and 0.5 ppb are obtained for all compounds in water and soil, respectively. Method development work for the extraction and cleanup of the analytes from water and soil was conducted at CIBA-GEIGY. Method validation and all LC/MS work was conducted at ALTA.

This method is restricted to use under the supervision of analysts experienced in the operation of TSP-LC/MS/MS.

Prepared by: Robert A. Bethem

Management Approval: Robert A. Bethem Date: 9-9-91

QA Officer Approval: Robert S. Peterson Date: 9-9-91

B. Principle

1. Water

A 100-mL aliquot of a water sample is made acidic with phosphoric acid and extracted with methylene chloride and then ethyl acetate. The organic extracts are evaporated to near dryness on a rotary evaporator and the residue is redissolved in 1.0-mL of acetonitrile. Just prior to analysis, a 200 ul sample aliquot is taken to near dryness and then re-diluted to 200 ul with 0.2M NH_4OAc. The extract is then analyzed for parent and four degradation products by high performance liquid chromatography/mass spectrometry (LC/MS). A second 100 ul aliquot is also taken, brought to complete dryness, re-diluted with 100 ul of 0.2M NH_4OAc, and analyzed for CGA-177288 by LC/MS on a different LC column in an acidic mobile phase. A flow chart for this process can be found in Figure 1 of this method.

2. Soil

Soil samples (20 g) are extracted at room temperature with mechanical shaking in 50-mL of 20% (v/v) methanol/phosphate buffer (0.03 M, pH adjusted to 6.0). After centrifugation, a 25-mL aliquot of the soil extract is diluted with water, acidified, and then extracted with methylene chloride and then with ethyl acetate. The organic extracts are evaporated to near dryness on a rotary evaporator and the residue is redissolved in 1.0-mL of acetonitrile (ACN). Just prior to analysis, a 200 ul sample aliquot is taken to near dryness and then re-diluted to 200 ul with 0.2M NH_4OAc. The extract is then analyzed by high performance liquid chromatography mass spectrometry (LC/MS). A second 100 ul aliquot is also taken, brought to complete dryness, re-diluted with 100 ul of 0.2M NH_4OAc, and analyzed by LC/MS with a different LC column in an acidic mobile phase. A flow chart for this process can be found in Figure 2 of this method.

C. Safety

The toxicity or carcinogenity of each reagent used in this method has not been precisely determined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of available material data handling sheets should be available to all personnel involved in the chemical analysis.

II. MATERIALS AND METHODS

A. Apparatus

1. Bottle, amber Boston round, appropriate size for storage of standard solutions.
2. Bottle, polypropylene, with cap, appropriate size for soil extractions.
3. Centrifuge, IEC Centra 8 (International Equipment Corporation) or equivalent.
4. Flask, round bottom, 500-mL.
5. Flask, Erlenmeyer, 250-mL.
6. Funnel, filter.
7. Funnel, separatory, 250-mL.
8. Glass wool (Fisher cat. #11-390) or equivalent.
9. Mechanical shaker, orbital (Fisher cat. #12-812) or equivalent.
10. pH stick, Corning (Fisher cat. #13-641-536) or equivalent.
11. Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent.
12. Tube, concentration/centrifuge, 50-mL (Fisher cat. #05-538-40B) or equivalent.
13. Syringe, 5-mL plastic, (BD #9603) or equivalent.
14. Filter, syringe, 0.45u (Gelman 4472 Acrodisc 3 CR PTFE) or equivalent.
15. Balance, Analytical, capable of weighing to the nearest 0.0001 g.
16. Vials, 1-mL (Waters 78514 or equivalent).
17. Vials, 4-mL (Waters 72710 or equivalent).
18. Low volume inserts (Waters 72704 or equivalent).
19. Caps with septa (Waters 73010 or equivalent).

B. Reagents

1. Acetone, Optima grade (Fisher cat. #A929-4) or equivalent.
2. Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.
3. Ethyl Acetate, HPLC grade (Fisher cat. #E195-4) or equivalent.
4. Methylene chloride, HPLC grade (Fisher cat. #D143-4) or equivalent.
5. Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.
6. Ammonium acetate, crystals, (EM Science cat. #AX1220-1) or equivalent.
7. Extraction solvent (soil): 20% (v/v) methanol/phosphate buffer.
8. HPLC mobile phase, 0.2M ammonium acetate. Dissolve 15.4 grams of ammonium acetate in 1 L of water.
9. Phosphate buffer, 0.03 M, pH = 6.0 ± 0.5. Dissolve 2.1 grams of sodium phosphate monobasic monohydrate in 500-mL of purified water. Adjust the pH to 6.0 ± 0.5 with phosphoric acid and sodium hydroxide.
10. Phosphoric acid, 85% (Conc.) (Fisher cat. #A242-1) or equivalent. 1% (v/v) Phosphoric acid/water.
11. Sodium hydroxide, reagent grade, 50% (w/w) (Fisher cat. #SS254-500) or equivalent.
12. Sodium phosphate monobasic, monohydrate, (Fisher cat. #S369-500) or equivalent.
13. Sodium sulfate, anhydrous (Fisher cat. #S421-3) or equivalent.
14. Water, distilled, HPLC grade, or purified in-house with a HYDRO™ purification system, or equivalent.
15. CGA-136872, CGA-191429, CGA-120844, CGA-177288, CGA-171683, and CGA-27913 analytical standards, CIBA-GEIGY Corp., P. O. Box 18300, Greensboro, NC 27419.

C. Analytical Procedure

1. Water

1.1 Weigh a 100 g aliquot of the water sample into a 250-mL separatory funnel. (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). Alternatively, measure out in a graduated cylinder 100-mL of the water sample and transfer to a 250-mL separatory funnel.

1.2 Add 2.0-mL of conc. phosphoric acid and shake to mix. The pH of the aqueous solution should be < 2.5. Sample fortification, if required, should be done at this time (refer to Section II.J.2.0).

1.3 Add 75-mL of methylene chloride. Shake for thirty seconds. Allow the two phases to separate. A smaller volume of extracting solvent may be used if satisfactory recoveries are demonstrated in the field matrix spikes.

1.4 Place a small glass wool plug into a filter funnel. Add approximately 50 g of sodium sulfate, and rinse with approximately 10-mL of methylene chloride. Drain the lower organic layer through the sodium sulfate into a 500-mL round bottom flask.

1.5 Extract the sample with a second 75-mL aliquot of methylene chloride. Follow the directions in Step 1.3 and collect the organic phase into the same 500-mL flask. Rinse the sodium sulfate with an additional 10-mL of methylene chloride and drain into the 500-mL flask.

1.6 Add 75-mL of ethyl acetate. A smaller volume of extracting solvent may be used if satisfactory recoveries are demonstrated in the field matrix spikes. Shake for thirty seconds. Allow the two phases to separate. Drain the lower aqueous phase into a 250-mL erlenmeyer flask. Drain the organic phase through the sodium sulfate (pre-rinsed with approximately 10-mL of ethyl acetate) into the 500-mL flask containing the organic extracts from Steps 1.3 and 1.4.

1.7 Pour the aqueous portion back into the separatory funnel, (rinsing the erlenmeyer with ethyl acetate), and extract the aqueous sample with a second 75-mL aliquot of ethyl acetate. Follow the directions provided in Step 1.6 and collect the organic into the same 500-mL flask.

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1.8 Rinse the separatory funnel with ethyl acetate draining through the sodium sulfate into the 500-mL flask. Rinse the sodium sulfate three times with approximately 10-mL of ethyl acetate each. Repeat this procedure using methylene chloride.

1.9 Remove the organic solvents from the sample by placing the 500-mL flask on a rotary evaporator with a water bath temperature of approximately 35°C. Remove the flask when approximately 5-mL remain. Note: Do not allow the extract to go to dryness in any of the concentration steps.

1.10 Transfer the extract to a 50-mL concentration tube, rinsing the round bottom 5 times with approximately 3-mL each of acetone.

1.11 Add approximately 2-mL of acetonitrile to the concentration tube.

1.12 Place the tube on a rotary evaporator (water bath temperature of approximately 35°C) and remove the solvent until just under 2-mL remains.

1.13 Rinse the walls of the tube with approximately 6-mL of acetonitrile.

1.14 Place the tube on the rotary evaporator (water bath temperature of approximately 35°C) and remove the solvent until just under 1-mL remains. Remove the tube from the evaporator.

1.15 Adjust the volume to 1.0-mL with acetonitrile.

1.16 Transfer the contents to a five-mL syringe with a 0.45 micron filter and collect the filtered extract into a 1-mL vial.

1.17 Store the acetonitrile extract at less than -5 degrees C until the day of analysis.

1.18 On the day of analysis for all analytes except CGA-177288, aliquot 200 ul of the extract into a 1-mL autosampler vial and concentrate to near dryness (meniscus at bottom of the 1-mL vial) with nitrogen. Adjust the volume back to 200 ul with 0.2M NH_4OAc.

1.19 Analyze by LC/MS using the HPLC conditions in Table I and LC/MS conditions in Table III.

1.20 On the day of analysis for CGA-177288, aliquot 100 ul of the extract into a 1-mL autosampler vial and concentrate to complete dryness with nitrogen. Alternatively, a 100 ul aliquot may be taken to dryness after step 1.16 and stored according to 1.17. Adjust the volume back to 100 ul with 0.2M NH_4OAc.

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1.21 Analyze by LC/MS using the HPLC conditions in Table II and LC/MS conditions in Table IV.

2. Soil

2.1 Weigh 20 g of soil sample into a 250-mL polypropylene centrifuge bottle. Sample fortification, if required, should be done at this time (refer to Section II.J.2.0).

2.2 Add 50-mL of the soil extraction solvent. Place the cap on the bottle. Place the bottle in an orbital shaker and shake the sample for two hours at room temperature, or 25°C if the shaker has a water bath.

2.3 Remove the sample from the shaker. Place the bottle in the centrifuge at approximately 4000 rpm for approximately 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.4 Add 75-mL of water, 2.0-mL of conc. phosphoric acid, and 25-mL of the supernatant from Step 2.3 to a 250-mL separatory funnel. Shake the contents to mix. The pH of the aqueous solution should be < 2.5.

2.5 The remainder of the cleanup procedure is identical to the procedure for water. At this point refer to Step 1.3 above (the methylene chloride extraction) and follow Steps 1.3 through 1.21.

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D. Instrumentation

1. Description and Operating Conditions - HPLC

See Table I for a description of the HPLC system and chromatographic conditions for CGA-27913, CGA-191429, CGA-120844, CGA-136872, and CGA-171683. See Table II for a description of the HPLC system and chromatographic conditions for CGA-177288.

2. Description and Operating Conditions - Mass Spectrometer

In order to achieve maximum sensitivity and specificity for these analytes, it has been necessary to operate the instrument in both positive and negative ionization modes. With the exceptions of CGA-27913 and CGA-171683, all data is collected using MS/MS techniques. There are two primary reasons for employing MS/MS technology.

- Thermospray ionization is a "soft" ionization technique, much like chemical ionization, and usually results in only one ion for detection. MS/MS induces additional fragmentation on this ion (CID) and, therefore, provides additional mass spectral information for confirmation of analyte identity.

- Due to the high chemical specificity of the technique, detector noise is dramatically reduced and signal to noise is enhanced. This in turn leads to lower detection limits and greatly reduces the occurrence of interferences.

CGA-27913 and CGA-171683 do not yield adequate product ions for analysis by MS/MS.

Standard mass spectrometer source tuning techniques are used for thermospray mass spectrometry. Mass calibration is based on the most recent FC-43 calibration by electron impact ionization. Mass calibration may be checked in TSP mode by checking background ions at m/z 59, m/z 187, and m/z 269. Normally, no calibration adjustments are necessary.

When the instrument is operated in the product ion mode (MS/MS), quantitation is based on the area of the product ion resulting from the collision induced dissociation (CID) of the protonated molecular ion or ammonium adduct ion (precursors).

During the course of the analytical run for primisulfuron-methyl and four of its degradation products, the instrument must be switched from negative ion (Q1MS), to positive ion MS/MS, and then back to negative ion (Q1MS). Switching times must be selected so that an adequate number of scans precede and follow each peak to be quantified. See Table III for a description of the scanning modes and switching times for the mass spectrometer.

The analysis for CGA-177288 is performed by negative ion MS/MS. See Table IV for a description of the thermospray and instrument operating conditions.

3. Description and Operating Conditions - Thermospray Interface

Extensive optimization studies were performed to determine the best operating parameters for each of the six analytes. In general, CGA-177288 and CGA-27913 give optimum response at high ion source temperatures, with the remaining compounds (especially CGA-120844) optimizing at cooler source temperatures. On the Finnigan TSP2 interface, a source temperature of 260° C. seems to provide the best results overall. The optimized values for the TSP vaporizer may vary with time. In general, the optimum vaporizer temperature is found to be approximately 15 degrees C. below the take off temperature as evidenced by the m/z 59 reagent ion. With the Finnigan TSP2 vaporizer with a sapphire tip, 100° C. is usually the best setting.

See Table III and Table IV for a description of the operating parameters for the thermospray interface and mass spectrometer.

4. Calibration and Standardization

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

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4.2 Calibrate the instrument by using the average response factor from detector response (chromatographic peak area) and the amount of analyte injected encompassing a range from 0.125 ng to 5 ng, (50-ul injections). If the standard deviation for the three point curve is less than 20%, then the method is considered to be linear. A broader concentration range for the three point curve may be used providing that the detector response remains linear.

4.3 Generally, analytes should be calibrated relative to the average response factor from the two preceding standards and one standard following. In any event, the three standards used must be of three different concentrations encompassing the range of calibration.

4.4 Sample screening for none detectable amounts may be employed without regards to the 20% RSD criteria, providing that the low standard (2.5 ng/mL) yields adequate signal to noise (greater than 5:1) for each analyte.

E. Interferences

1. There are no known interferences originating from the sample cleanup procedure.

2. Interferences have been observed for CGA-27913 at concentrations less than the reporting limit for soil and water.

F. Confirmatory Techniques

1. Although no alternative techniques exist, this method provides detection based on highly specific negative ion or MS/MS techniques. In addition, retention time data is also available for confirmation.

G. Time Required

1. The sample cleanup procedure can be completed for a set of eight samples in ten hours.

2. Each LC/MS analysis for parent and four degradation products requires 20 minutes.

3. Each LC/MS analysis for CGA-177288 requires 5 minutes.

H. Modifications and Potential Problems

1. Degradation of CGA-120844 is observed in aqueous solutions within a relatively short time (less than 72 hours) Therefore, extracts are stored in 100% acetonitrile in the freezer until the day of analysis. Prior to analysis, an aliquot of the extract is taken to near dryness and re-dissolved in 0.2M NH_4OAc.

2. Stock solutions must be prepared in 100% acetonitrile and stored in the freezer. LCMS analytical standards should be prepared in the initial mobile phase from the stock solution every 72 hours or sooner.

3. CGA-171683 will only partition into methylene chloride. CGA-177288 and CGA-27913 do not partition quantitatively into methylene chloride and require the additional ethyl acetate extractions.

4. Due to the volatility of CGA-171683, close supervision of samples is required during concentration steps. The evaporation techniques used for the sample aliquots prior to analysis is critical. Autosampler vials (1-mL) are used for both analyses. For CGA-177288, the 100 ul aliquot must be taken to complete dryness in order to avoid signal enhancement during analysis. For the parent analysis, the 200 ul sample aliquot must not be taken down beyond 20 ul or the recovery for CGA-171683 will drop below 50% In a 1-mL vial, 20 ul remains when the meniscus just touches the bottom of the vial.

5. The suggested operating temperatures were developed for a Finnigan TSP2 source as a result of extensive optimization studies. In general, those analytes run by negative ion optimize at "hot" source temperatures, and those run by positive ion optimize at "cool" temperatures. The suggested temperatures are therefore set at an intermediate value.

6. Enhancement of signal has been observed for CGA-120844, CGA-177288, and to a much lesser degree, the parent compound. For CGA-177288, taking the extract to complete dryness and injecting into an acidic mobile phase has corrected the problem for most analyses. Recoveries for CGA-120844 were observed to be as high as 151% during water method validation.

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I. Preparation of Standard Solutions

1. A 1 mg/mL stock solution for each analyte is prepared by dissolving 25.0 mg of pure material into 25-mL of acetonitrile. If the compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Transfer the stock standard solution into an amber bottle and seal with teflon lined caps only. Store the standards at -10°C or colder and protect from light when not in use. Stock standard solutions must be replaced after 1 year or sooner if comparison with check standards indicates a problem.

2. Using the 1 mg/mL stock solutions from Step 1, prepare a 10 ug/mL mixed standard containing all six compounds in acetonitrile. Replace the analytical solution every six months or sooner.

3. Fortification standards are prepared by dilution of the stock solution (Step 1.0) with acetonitrile. Fortification standards should be prepared such that no more than 2-mL of the solution is added to a sample. (Example: for a 100-mL water sample, the addition of 1.0-mL of a 0.5 ng/μL standard results in a fortification level of 5.0 ppb.)

4. LCMS analytical standards must be made fresh every 72 hours or sooner in the initial HPLC mobile phase. Standards should be prepared such that at least one is lower than the desired screening level and one is higher than the highest expected amount. Typically, a 10 ug/mL (ACN) stock solution is used for making the daily injection standards. (Example: for a 100 ng/mL standard, 100 ul of the stock solution is diluted to 10-mL in the initial mobile phase. Serial dilutions of 1:10, and 1:4 are then made for the 10 ng/mL and 2.5 ng/mL standards. Injection standards and fortification standards should be traceable to the same stock solution. Some record of the date that injection standards were diluted into the aqueous mobile phase must be maintained. If possible, this notation may be made directly into each standard data file header.

J. Determination of Sample Residues

1. Samples

1.1 Inject the sample solution from Step II.C.1.18 into the HPLC. The sample solution may be diluted with the sample diluent if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (ng/ml) is determined by multiplying the value of the chromatographic peak area by the average response factor (II.D.4.2).

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2. Fortified Samples

The method is validated 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 (water) or 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) to 100-mL of a water sample prior to any of the cleanup steps. (Note: A sample size smaller than 100-mL may be used, but this will increase the determination limit in ppb.) The total volume of the added fortification solution should not exceed 2.0-mL (example: add 1.0-mL of a 0.5 ng/μL fortification solution to 100-mL of a water sample to fortify at 5.0 ppb.) For soil samples, fortify 20 g of the sample and allow sufficient time for the fortification solvent to evaporate before proceeding to Step 2.2

2.2 Proceed with the sample cleanup procedure.

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3. Calculations

3.1 Calculate the response factor (in ng/ml) from equation (1):

$$RF(ng/ml) = \frac{Conc_{STD}}{Area_{STD}}$$

Where: $Area_{STD}$ = the area response from a standard injection

$Conc_{STD}$ = concentration of the injected standard (ng/ml)

3.2 Quantitation of samples will be performed based on the average response for the standards immediately preceding and following the samples and is defined as follows:

$$RF_{AVG} = \frac{(RF_{STD1}) + (RF_{STD2}) + (RF_{STD3})}{3}$$

Where: RF_{STD1} = the response factor from the first standard

RF_{STD2} = the response factor from the second standard

RF_{STD3} = the response factor from the third standard

Normally, RF_{STD1} and RF_{STD2} will precede the sample and RF_{STD3} will follow.

The per cent relative standard deviation for RF_{avg} must be less than 20%. If the instrument response falls outside of this range after two re-injections of the standard, then the affected samples must be re-injected. In the instance of screening samples where no positive detections are made, it is only necessary to demonstrate adequate system sensitivity via injection of the low calibration standard.

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3.3 Sample calculations are done according to the following formula:

$$Amount_{SAMP} = \frac{(Area_{SAMP})(RF_{AVG})(FV)}{(Wt_{SAMP})\ (Df)}$$

And,

3.4

$$Amt_{SAMP} = \frac{(Area_{SAMP})(RF_{AVG})}{(Cf)\ (Df)}$$

Where: Amt_{SAMP} = Final Sample Amount (ppb),

$Area_{SAMP}$ = Area response from unknown,

RF_{AVG} = Response Factor (ng/ml),

FV = Final Volume (ml),

Wt_{SAMP} = Sample weight (g),

Cf = Concentration factor (Wt_{SAMP}/FV),

Df = Dilution factor (Vol_{init}/Vol_{fin}).

3.5 If the sample amount calculated from the above equation is greater than the screening level for soils or waters, then one or more of the following additional calculations are made:

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3.6 Soil or water residue values are corrected for recoveries less than 100% according to the following formula:

$$Amount_{REC} = \frac{Amount_{SAMP}\ (ppb)}{\frac{R\ (\%)}{100}}$$

3.7 Soil residue values are corrected for recoveries less than 100 % and/or percent moisture:

$$Amount_{DryWt} = \frac{Amount_{SAMP}\ (ppb)}{(M)}$$

$$Amt_{DryWt,\ REC} = \frac{Amt_{SAMP}\ (ppb)}{(\frac{R\ (\%)}{100})\ (M)}$$

Where: Amt_{DryWt} = Final Sample Amount percent moisture corrected

Amt_{REC} = Recovery corrected for Sample Amount

$Amt_{DryWt,REC}$ = Final Sample Amount corrected for average recoveries and percent moisture,

R = Average Recovery of fortified control sample(s) where R is less than 100 %,

M = Moisture content correction factor (DryWt/WetWt).

3.8 If $Amount_{SAMP}$ is less than the reporting limit then the results are reported as less than this amount and all other calculations are reported as "NC" (not calculated). Field sample results greater than twice the screening limit are reported to two significant figures. All other field sample results exceeding the screening limit are reported to one significant figure.

3.9 The results from fortified control samples are reported to three significant figures if the spike level is greater than twice the screening limit. All other spike results are reported to two significant figures. Percent recoveries are reported to two significant figures if the result is less than 100% and three significant figures if the result is greater than 100%.

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TABLE I. HPLC OPERATING CONDITIONS FOR THE DETERMINATION OF PRIMISULFURON-METHYL (CGA-136872) AND FOUR METABOLITES BY TSP-LC/MS/MS

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent)

Waters 590-MS HPLC isocratic pump (or equivalent)

Waters WISP 712 autosampler (or equivalent)

Operating Conditions:

Column:	Nova-Pak, 4 um, 4.0 mm x 15 mm HPLC column, Waters part # 86344
Mobile Phase:	10% (v/v) acetonitrile/(water with 0.2M NH_4OAc), step gradient to 30/70 at 0.1 minute, step gradient to 55/45 at 3.5 minutes, step gradient to 100% ACN at 8 minutes, re-equilibrate at 12 minutes.
Flow Rate:	1.2 ml/min
Injection Volume:	50 ul
Cycle Time:	20 minutes

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TABLE II. HPLC OPERATING CONDITIONS FOR THE DETERMINATION OF CGA-177288 BY TSP-LC/MS/MS

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent)

Waters 590-MS HPLC isocratic pump (or equivalent)

WISP 712 autosampler (or equivalent)

Operating Conditions:

Column:	Two Zorbax Rx-C8 Reliance columns, (4mm x 80 mm 5 micron), (MAC MOD part # 820967-901) in series using fitting kit (MAC MOD part # 820678-901).
Mobile Phase:	Isocratic at 3% (v/v) acetonitrile/(1% HOAc with 0.2M NH_4OAc).
Flow Rate:	1.2 ml/min
Injection Volume:	50 ul
Cycle Time:	5 minutes

ALTA

TABLE III. TSP-LC/MS/MS OPERATING CONDITIONS FOR THE DETERMINATION OF PRIMISULFURON-METHYL (CGA-136872) AND FOUR METABOLITES BY TSP-LC/MS/MS

Instrumentation:

Mass Spectrometer: Finnigan MAT TSQ-700 capable of positive and negative ion MS and/or MS/MS and equipped with a TSP2 thermospray interface (or equivalent).

Operating Parameters:

Collision gas pressure: 2.5 mtorr (2.0 - 3.0 mtorr)

Collision offset: -18 volts (-10 - -20 volts, positive ion)
18 volts (10 - 20 volts, negative ion)

Vaporizer Temperature: 100 degrees C (90 - 110 degrees C)

Source Temperature: 260 degrees C (220 - 300 degrees C)

Repeller Voltage: 40 volts (0 - 50 volts, positive)
-20 volts (0 - -50 volts, negative)

Analyte	MW	Analysis Mode	Ions monitored (precursor ions)	Retention Time (min.)	Detection Limit
CGA-136872	468	MS/MS (+) 3 - 7.1 min.	(271) - 160 (233) - 199	6:30	10 pg
CGA-191429	454	MS/MS (+) 3 - 7.1 min.	(271) - 160	3:50	25 pg
CGA-120844	215	MS/MS (+) 3 - 7.1 min.	(233) - 199	4:00	10 pg
CGA-27913[1]	183	MS (-) 1 - 3 min.	182	1:40	25 pg
CGA-171683[1]	227	MS (-) 7.1 - 10 min.	226	8:40	25 pg

[1] This analyte is scanned in the Q1MS mode with the collision gas on.

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TABLE IV. TSP-LC/MS/MS OPERATING CONDITIONS FOR THE DETERMINATION OF CGA-177288 BY TSP-LC/MS/MS

Instrumentation:

Mass Spectrometer: Finnigan MAT TSQ-700 capable of negative ion MS/MS and equipped with a TSP2 thermospray interface (or equivalent).

Operating Parameters:

Collision gas pressure: 2.5 mtorr (2.0 - 3.0 mtorr)

Collision offset 18 volts (10 - 20 volts)

Vaporizer Temperature: 100 degrees C (90 - 110 degrees C)

Source Temperature: 260 degrees C (220 - 300 degrees C)

Repeller Voltage: -20 volts (0 - -50 volts)

Analyte	MW	Analysis Mode	Ions monitored (precursor ions)	Retention Time (min.)	Detection Limit
CGA-177288	201	MS/MS (-) 1.3 - 3.2 min.	(200) - 92	2	25 pg

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FIGURE 1.

FLOW DIAGRAM FOR THE DETERMINATION OF CGA-136872 AND METABOLITES IN WATER

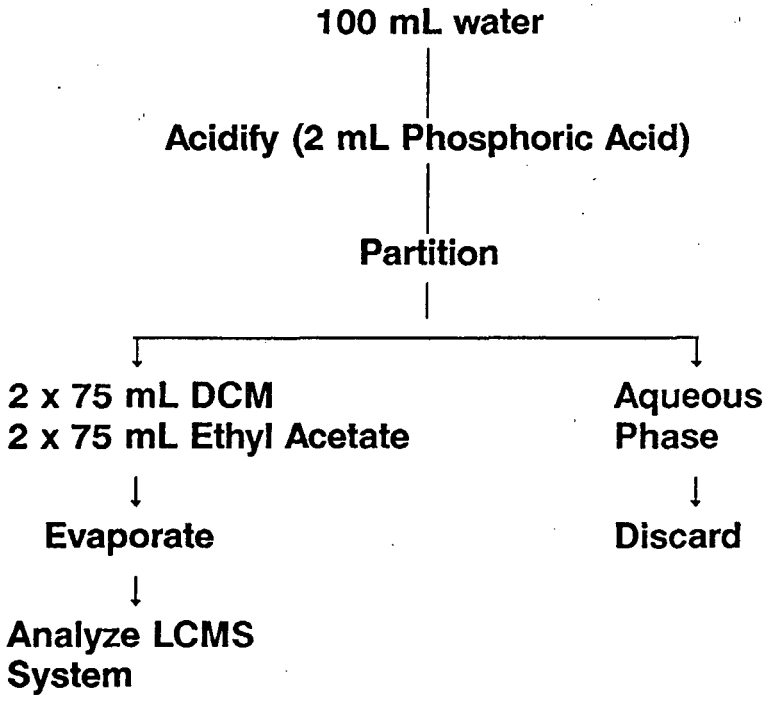

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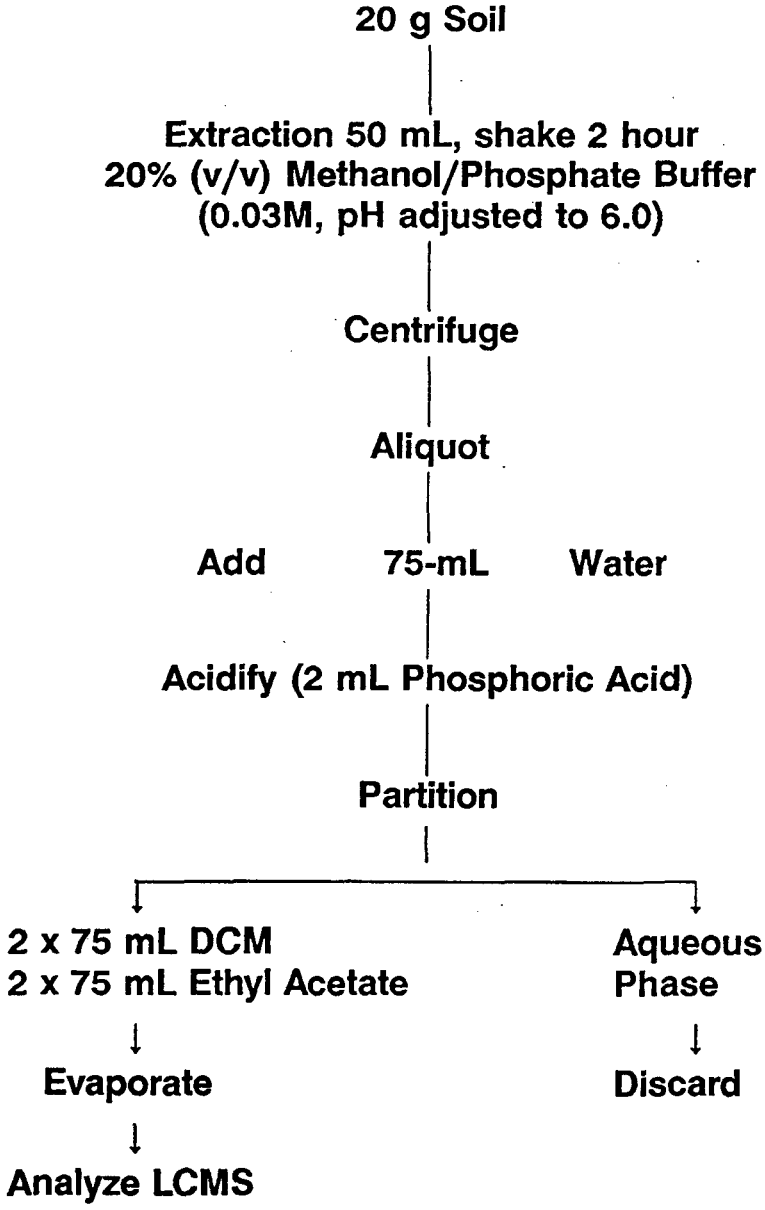
FIGURE 2.

FLOW DIAGRAM FOR THE DETERMINATION OF CGA-136872 AND METABOLITES IN SOIL



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APPENDIX 3 Syngenta Analytical Method CIGPSM1-Modifications

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.

Stock Solutions

Prepare a 100 µg/mL stock solution for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 by one of the following methods.

Weigh out accurately, using a five figure balance, 10.00 mg (corrected for impurity) of each analytical standard into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with acetonitrile to give 100 µg/mL stock solution of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288.

Note: The stock solutions must be stored frozen with extremely cautions.

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 acetonitrile 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.

Preparation of Fortification Standard Solutions

Prepare an intermediate standard solution containing CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 (1 µg/mL) by mixing 1 mL of each stock solution (Section 2.3.1) with 99 mL of MeOH0 in a volumetric flask (100 mL) and diluting to the mark. Prepare the first level fortification standard (0.1 µg/mL) by mixing 5 mL of the intermediate standard solution (1 µg/mL) with 45 mL of MeOH in a 50 mL volumetric flask. Prepare the second level fortification standard (0.01 µg/mL) by mixing 5 mL of the first level fortification

standard (0.1 µg/mL) with 45 mL of MeOH in a 50 mL volumetric flask. It is strongly recommended that such two fortification standard solutions are prepared and used for fortification.

Preparation of Calibration Standards for LC-MS/MS

Non Matrix Match Calibration Standard Solutions

Prepare the calibration standard solutions by serial dilutions of the first level fortification standard solution (0.1 µg/mL) with 0.1% acetic acid in ultra-pure water. For example, transfer 1 mL of the 0.1 µg/mL fortification standard into a volumetric flask (100 mL) and mix with 99 mL of 0.1% acetic acid in ultra-pure water to the mark to yield 1 ng/mL calibration standard containing CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. Dilute the calibration standard solution further with 0.1% acetic acid in ultra-pure water to yield lower concentrations. It is strongly recommended that the following calibration standards are prepared: 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 for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in 0.1% acetic acid in ultru pure water.

Matrix Match Calibration Standard Solutions

Matrix matched standards are needed for this method if significant matrix effect is observed. In case matrix match standard solutions are needed, the first level fortification standard (0.05 µg/mL) is used to prepare intermediate standards at 0.1 ng/mL, 0.2 ng/mL, 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/mL and 10 ng/mL for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in 0.1% acetic acid in ultra-pure water. Acidify the matrix ground water control sample with acetic acid to 0.1% level. Mix 0.1 mL µL of each intermediate standard with 0.9 mL of the acidified matrix control ground water sample (Section 3.5) to yield 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 matrix match standard solutions.

Calibration Curves

Calibration curves should be constructed for quantitation of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in unknown samples. At least five levels of calibration standard solutions over an appropriate concentration range should be prepared and a weighing factor of 1/x should be used.

Standard Solution Storage and Expiration

Stock solutions must be stored frozen when not used. All standard solutions should be stored in a refrigerator when not in use to prevent decomposition and/or concentration of the standards. Standard solutions should be allowed to equilibrate to room temperature prior to use. Note: Check the injection standard stability against the fortification standards from time to time.

An expiration date of one week for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 is recommended unless additional data are generated to support a longer expiration date.

ANALYTICAL PROCEDURE

Precautions

a) Bottled HPLC grade ultra-pure water is used to prepare the LC mobile phase, 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 (0.5 ppb) and samples with expected residues greater than 1 ppb (µg/L) should be diluted with 0.1% acetic acid in ultra-pure water so that the final analyte concentration does not exceed 1 ppb (µg/L). It may also be useful to include blank injections of 0.1% acetic acid in ultra-pure water after high level samples to clear any observed carry-over greater than 10% of the LOQ;

Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis. If water samples are received frozen, they should be allowed to defrost thoroughly before use. Water samples should be stored in the darkness in plastic containers rather than glass to prevent losses of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 due to adsorption or photo degradation.

Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), recovery samples should be included with each sample set. To each pre-measured control water sample (20 mL), add 100 µL of the combined fortification standard containing CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. At least one untreated control and two recovery samples should be analyzed with each sample set. For example, to prepare a recovery sample at LOQ (0.05 ppb), transfer 20 mL of the untreated sample (control) into a glass graduated cylinder (50 mL) and add 100 µL of the second level (0.01 µg/mL) fortification standard to the sample. Do not add <0.1 mL or > 1 mL of fortification standard to samples.

Extraction

A summary of the method is included in flow-chart form in Appendix 3.

a) Measure a representative amount of water sample (20 mL) into a polypropylene centrifuge tube (50 mL). Fortify untreated control samples, if needed, with known amount of the combined fortification standard solutions;

b) Add 20 µL of concentrated acetic acid to the sample and mix well to acidify the sample.

Final Fraction

a) Stopper the polypropylene centrifuge tube (50 mL) and shake the sample vigorously for 10 seconds to yield sample final fraction. Transfer an aliquot (~1.5 mL) from the sample final fraction into a suitable autosampler vial ready for final determination by LC-MS/MS. Filter the sample with 0.45µm Nylene filter, if particles are visible.

FINAL DETERMINATION

The following instrumentation and liquid chromatographic conditions are suitable for analysis of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. 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.

Instrument Description

Pump	:	Waters Acquity UPLC® system (I Class) with Sample Manager and Column Manager
Detector	:	Applied Biosystems Sciex API 4000 triple quadrupole mass spectrometer with Analyst TM software version 1.6.2

Chromatographic Conditions

Column	:	Atlantis® T3 100 mm x 3.0 mm, 5 µm
Column Oven Temperature	:	40°C
Injection volume	:	100 µL
Stop Time	:	14 minutes
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: 0.1 mM ammonium acetate aqueous solution Solvent 2: MeOH

Mobile Phase Composition

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0	85	15	0.6
1	85	15	0.6
5	50	50	0.6
8	5	95	0.6
11	5	95	0.6
11.1	85	15	0.6
14	85	15	0.6

Note: Under these conditions the retention times of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 are 7.2 minutes, 6.4 minutes, 4.4 minutes, 3.0 minutes, 7.5 minutes and 2.1 minutes.

Column Switching Valve Program

Time (min)	Valve Position
	To waste
1.5	To mass spectrometer

Notes: The column eluate may be diverted to waste for the first 1.5 minutes to prevent ionic material from the sample contaminating the mass spectrometer front plate, if required. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary. It is not necessary to reduce the flow rate into the mass spectrometer when using the API 4000.

Mass Spectrometer Conditions for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288

Interface	:	TurboIonSpray			
Polarity	:	Negative			
Curtain gas (CUR)	:	Nitrogen set at 25 (arbitrary units)			
Temperature (TEM)	:	550°C			
Ion spray voltage	:	-4200			
Collision gas setting (CAD)	:	Nitrogen set at 12 (arbitrary units)			
Gas 1 (GS1)	:	Air set at 55 (arbitrary units)			
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)			
Interface heater (ihe)	:	On			
Scan type	:	MRM			
MRM Conditions		CGA136872 primary transition	CGA136872 confirmatory transition	CGA191429 primary transition	CGA191429 confirmatory transition
Q1 *m/z*	:	467.2	467.2	453.0	453.0
Q3 *m/z*	:	225.8	176.0	156.0	92.1
Dwell time	:	200 ms	200 ms	200 ms	200 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-42 V	-42 V	-40	-40
Entrance potential (EP)	:	-10 V	-10 V	-10	-10
Collision energy (CE)	:	-19 V	-42 V	-38	-66
Collision cell exit potential (CXP)	:	-10 V	-10 V	-7	-6

MRM Conditions		CGA120844 primary transition	CGA120844 confirmatory transition	CGA27913 primary transition	CGA27913 confirmatory transition
Q1 *m/z*	:	213.9	213.9	181.9	181.9
Q3 *m/z*	:	182.0	106	106.0	42.0
Dwell time	:	200 ms	200 ms	200 ms	200 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-25 V	-25 V	-65	-65
Entrance potential (EP)	:	-10 V	-10 V	-10	-10
Collision energy (CE)	:	-13 V	-32 V	-26	-45
Collision cell exit potential (CXP)	:	-7 V	-5 V	-6	-5

MRM Conditions		CGA171683 primary transition	CGA171683 confirmatory transition	CGA177288 primary transition	CGA177288 confirmatory transition
Q1 *m/z*	:	226.0	226.0	200.0	200.0
Q3 *m/z*	:	175.8	125.9	155.8	92.0
Dwell time	:	200 ms	200 ms	200 ms	200 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-45 V	-45 V	-34	-34
Entrance potential (EP)	:	-8 V	-10 V	-10	-9
Collision energy (CE)	:	-18 V	-34 V	-16	-28
Collision cell exit potential (CXP)	:	-10 V	-9 V	-11	-5

APPENDIX 6 Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. LIMS was used to calculate the ppb and percent recovery and presented in Microsoft® Excel. The following equations were used for quantitation:

The following equations are used for residue calculations within MassHunter:

a) Calibration curve m : Solving for x: $\frac{-}{m}$

Where, m = Slope
b = y-intercept
x = Amount found (ng)
y = Peak area

b) Amount of sample in e ted mL) $\frac{\text{Sample amt. mL)} \times \text{In . si e mL)}}{\text{inal sample ol. mL)}}$

c) Anal te on entration pp) $\frac{\text{Amount found ng)}}{\text{Amount of sample in e ted mL)}}$

d) Per ent re o e r $\left(\frac{\text{pp i n sample} - \text{pp i n ontrol}}{\text{pp a dded}}\right) \times 100$

As an example, calculations to obtain the percent recovery in control water sample from WO-14030501fortified with primisulfuron-methyl in lab code 14030501-Recovery1-1. The calculations are shown below:

a) Calibration curve: 4408077.855169) − 16343.557146

Solving for x: $\frac{34991 \quad 16343.557146}{4408077.855169} = 0.00423 \text{ ng}$

b) Amount of sample in e ted mL) $\frac{20.0 \text{ mL} \times 0.08 \text{ mL}}{20.0 \text{ mL}} = 0.08 \text{ mL}$

c) Anal te on entration pp) $\frac{0.00423 \text{ ng}}{0.08 \text{ mL}} = 0.0529 \text{ ppb}$

Average residue found in the untreated sample (lab code: 130909002-002F and 130909002-002G) = 0.0000 ppb

d) Per ent re o e r $\left(\frac{0.0529 \text{ pp} - 0.0000 \text{ pp}}{0.05 \text{ pp}}\right) \times 100 = 105.8\%$