1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM070.01A is designed and developed in order to analyze CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in ground water system. The chemical structures and properties are presented in Figure 1. This method is based on the Environmental Chemistry Method CIGPSM1 (Reference 1) with modifications to the LC-MS/MS parameters, analytical column and cleanup procedures. In this new method, soil analysis is not included. The limit of quantification (LOQ) is set at 0.05 ppb (0.05 µg/L) for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in ground water systems.

This method satisfies with US EPA guidelines EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 Rev 4 and SANCO/825/00 Rev 8.1.

1.2 Method Summary

A portion of sub-sample (20 mL) is transferred into a Polypropylene centrifuge tube (50 mL) and acidified with acetic acid. The acidified sample is directly injected onto a LC-MS/MS system and analyzed for the residues of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. The limit of quantitation (LOQ) of this method is 0.05 ppb (0.05 µg/L) for the analytes in ground water system.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. 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 2.

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.

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 (1 µg/mL) standard solution containing CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 by mixing 1 mL of each stock solution (Section 2.3.1) with 94 mL of MeOH in a volumetric flask (100 mL) and diluting to the mark. Prepare the first level combined 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 combined fortification standard (0.01 µg/mL) by

mixing 5 mL of the first level combined fortification standard (0.1 µg/mL) with 45 mL of MeOH in a 50 mL volumetric flask. It is strongly recommended that such two combined 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 combined calibration standard solutions by serial dilutions of the first level combined fortification standard solution (0.1 µg/mL) with 0.1% acetic acid in ultrapure water. For example, transfer 1 mL of the 0.1 µg/mL combined fortification standard into a volumetric flask (100 mL) and mix with 99 mL of 0.1% acetic acid in ultrapure 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 ultrapure 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 ultrapure water.

Matrix-Matched Calibration Standard Solutions

Matrix-matched standards are needed for this method if significant matrix effect is observed. In case matrix-matched standard solutions are needed, the first level combined fortification standard (0.1 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 ultrapure water. Acidify the control water sample with acetic acid to 0.1% level. Mix 0.1 mL µL of each intermediate standard with 0.9 mL of the acidified control 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-matched 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.

2.4 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.

2.5 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 2).

Solvent and Reagent Hazards

	Acetonitrile	Methanol	Acetic Acid
Harmful Vapour	✓	✓	✓
Highly Flammable	✓	✓	X
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
OES Short Term (mg/m^3)	105	310	N/A
OES Long Term (mg/m^3)	70	260	N/A

N/A not known

At present there are insufficient data available to assign a Syngenta Hazard Classification for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. They 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 vapour. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

3.1 Precautions

a) Bottled HPLC grade ultrapure 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 ultrapure

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 ultrapure water after high level samples to clear any observed carry-over greater than 10% of the LOQ.

3.2 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 photodegradation.

3.3 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.

3.4 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.

3.5 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. Centrifuge the sample, if particles are visible.

3.6 Time Required for Analysis

The methodology is normally performed with a batch of 60 samples. One skilled chemist can complete the analysis of 60 samples in one day (8 hour working period).

3.7 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks 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 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. For example, an independent laboratory validation (ILV) study used Agilent LC and MS/MS to validate this method (Reference 3) and the relevant information and LC-MS/MS conditions are presented in Appendix 6.

4.1 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 2 software version 1.6.2

4.2 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	:	Analyze calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: 0.1 mM ammonium acetate in ultrapure water 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, 4.6 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.

4.3 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
Ionspray 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

Typical chromatograms for water are shown in the Figures Section. **Since the MS/MS sensitivity is extremely important for this method, see Appendix 4 for mass spectrometer parameters and tuning details and Appendix 5 for the profiles.**

4.4 Alternative Mass Spectrometer Conditions

Alternative MS/MS parameters and MEM transitions were used during the method ILV study (Reference 3) using an Agilent MS/MS instrument. These alternative parameters and MRM transitions are listed in Appendix 6.

4.5 Confirmatory Procedures

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

The concentrations of each analyte in unknown samples may be calculated in ppb (µg/L) for each sample as follows:

a) Prepare combined 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 peak areas corresponding to CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288. 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 (peak areas), x is the standard concentration (ng/mL) injected, 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) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where y is the instrument response value (peak areas) , x is the standard concentration (ng/mL) injected and a, b, c are constants.

f) Calculate the CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 residues in the sample, expressed as ppb (µg/L) as follows:

$$\text{Residue (ppb)} = \frac{\text{analyte concentration found (ng/mL)}}{\text{sample matrix concentration in final fraction (mL/mL)}}$$

Where analyte concentration found (ng/mL) is calculated from the standard calibration curve and sample matrix injected on column (mL/mL) is the sample matrix concentration in final fraction.

g) 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:

$$\text{Recovery} = \frac{\text{(ppb found in recovery sample - ppb found in control) x 100\%}}{\text{ppb fortified in recovery sample}}$$

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

$$\text{Corrected Residue (ppb)} = \frac{\text{residue (ppb)}}{\text{average percentage recovery}}$$

5.2 Single-Point Calibration Procedure

CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 may be calculated in ppb (µg/L) 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, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4.0. When a consistent response is obtained, measure the peak areas obtained for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288;

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288;

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

d) Calculate CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in the sample, expressed as ppb (µg/L) using a mean standard response from each of the injections bracketing the sample as follows:

$$\text{Residue (ppb)} = \frac{\text{Peak area (SA)}}{\text{Peak area (STD)}} \times \frac{\text{Standard concentration (ng/mL) injected}}{\text{Matrix sample concentration in final fraction (mL/mL)}}$$

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 4).

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed as detailed in Sections 3.0 and 4.0 with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analyzed with each set of samples.

At least two recovery samples (control samples accurately fortified with known amounts of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 should also be analyzed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found, if necessary. The fortification levels should be appropriate to the unknown analyte concentrations expected.

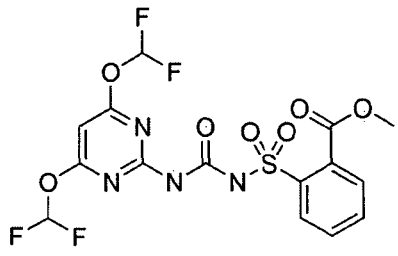
Recovery efficiency is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq$20%.

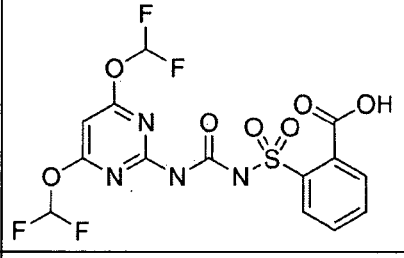
Note: When the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

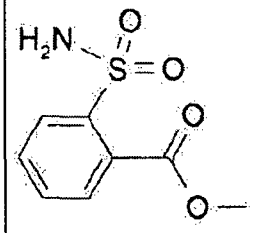
7.0 SPECIFICITY

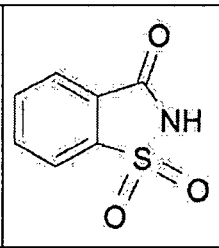
It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

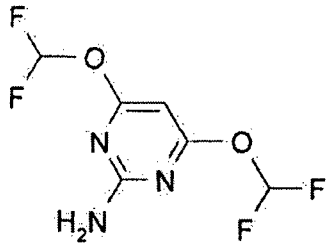
FIGURE 1 Chemical Structures

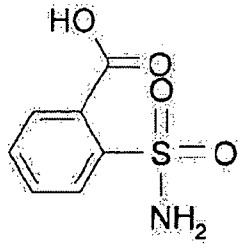
Compound	
Common Name:	Primisulfuron Methyl
Code Name:	CGA136872
IUPAC Name:	2-{3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl)-ureidosulfonyl}benzoic acid methyl ester
CAS Number:	113036-87-6
Molecular Formula:	$C_{14}H_{10}F_4N_4O_7S$
Molecular Weight:	468.3
Source:	Syngenta Product Safety Greensboro---Logistics and Support

Compound	
Common Name:	None
Code Name:	CGA191429
IUPAC Name:	2[4.6-bis(difluoromethoxy)-pyrimidin-2-yl] aminocarbonyl-aminosulonylbenzoic acid
CAS Number:	Not registered
Molecular Formula:	$C_{13}H_8F_4N_4O_7S$
Molecular Weight:	454.3
Source:	Syngenta Product Safety Greensboro---Logistics and Support

Compound	
Common Name:	None
Code Name:	CGA120844
IUPAC Name:	methyl-2-[aminosulfonyl]benzoate
CAS Number:	Not registered
Molecular Formula:	$C_8H_9NO_4S$
Molecular Weight:	215
Source:	Syngenta Product Safety Greensboro---Logistics and Support

Compound	
Common Name:	None
Code Name:	CGA27913
IUPAC Name:	1,3-benz-isothiazol-3(2H)-one,1,1,-dioxide
CAS Number:	Not registered
Molecular Formula:	$C_7H_5NO_3S$
Molecular Weight:	183
Source:	Syngenta Product Safety Greensboro---Logistics and Support

Compound	
Common Name:	None
Code Name:	CGA171683
IUPAC Name:	4,6-bis[difluoromethoxy]-2-aminopyrimidine
CAS Number:	Not registered
Molecular Formula:	$C_6H_5F_4N_3O_2$
Molecular Weight:	227
Source:	Syngenta Product Safety Greensboro---Logistics and Support

Compound	
Common Name:	None
Code Name:	CGA177288
IUPAC Name:	2-[amino-sulfonyl]benzoic acid
CAS Number:	Not registered
Molecular Formula:	$C_7H_7NO_4S$
Molecular Weight:	201
Source:	Syngenta Product Safety Greensboro---Logistics and Support

APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General glassware	General glassware	www.thermofisher.com/global/en/home.asp
Polypropylene Centrifuge Tube	50 mL capacity	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
Crimp cap autosampler vials and caps	2 mL capacity	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
LC-MS/MS system	API 4000 equipped with a TurboIonSpray source	www.AppliedBiosytems.com
HPLC system	Waters UPLC I-Class	www.waters.com
Autosampler	Waters UPLC I-Class	www.waters.com
HPLC column	Atlantis T3 100 mm x 3.0 mm, i.d., 5 µm particle size	www.agilent.com

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Ultrapure water	Optima® LC/MS	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
Ammonium acetate	ACS Certified	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
MeOH	Optima® LC/MS	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
Acetic Acid, Glacial	ACS Certified Plus	available from Thermal Fisher Scientific, Liberty Lane, Hampton, NH 03842
CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 analytical standards	GLP certified	Product Safety, Syngenta Crop Protection, LLC. Box 18300, Greensboro, NC 27419-8300.

Preparation of reagents

1. 0.1% acetic acid in ultrapure water: Add 1 mL concentrated acetic acid into 999 mL ultrapure water in a 1 L volumetric flask. Stopper the flask securely and shake to mix thoroughly

2. 0.1 mM ammonium acetate in ultrapure water: Add 7.1 mg of ammonium acetate in 1000 mL of ultrapure water in a 1 L volumetric flask and mix well.

APPENDIX 3 Method Flowchart

Measure 20 mL portions of water sample into a 50 mL polypropylene centrifuge tube

↓

Acidify to pH4 ±1 with 20 µL concentrated acetic acid

↓

Vial up and analyzed by LC-MS/MS

APPENDIX 4 LC-MS/MS Tuning Procedure

Calibration of instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288

(1) Infuse a standard solution of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 (1.0 µg/mL fortification standard solution) by an infusion pump into the UPLC column outlet line to the mass spectrometer interface via a T joint connector at a rate at of approximately 20 µL/min.

(2) Turn on the UPLC pump with an isocratic condition (90% 0.1% acetic acid and 10% MeOH) through the column specified in the method at a flow rate of 0.5 mL/min to the mass spectrometer interface.

(3) Using the instrument Analyst software quantitative negative mode optimization routine, tune the instrument for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288, ensuring that the correct ions are selected, i.e.:

CGA136872, initial Q1 *m/z* = 467.2 and product ion *m/z* = 225.8 for primary transition and initial Q1 *m/z* = 467.2 and product ion *m/z* = 176.0 for confirmatory transition;

CGA191429, initial Q1 *m/z* = 453.0 and product ion *m/z* = 156.0 for primary transition and initial Q1 *m/z* = 453.0 and product ion *m/z* = 92.1 for confirmatory transition;

CGA120844, initial Q1 *m/z* = 213.9 and product ion *m/z* = 182.0 for primary transition and initial Q1 *m/z* = 213.9 and product ion *m/z* = 106.0 for confirmatory transition;

CGA177288, initial Q1 *m/z* = 200.0 and product ion *m/z* = 92.0 for primary transition and initial Q1 *m/z* = 200.0 and product ion *m/z* = 155.8 for confirmatory transition;

CGA171683, initial Q1 *m/z* = 226.0 and product ion *m/z* = 175.8 for primary transition and initial Q1 *m/z* = 226.0 and product ion *m/z* = 125.9 for confirmatory transition;
CGA27913, initial Q1 *m/z* = 181.9 and product ion *m/z* = 106.0 for primary transition and initial Q1 *m/z* = 181.9 and product ion *m/z* = 42.0 for confirmatory transition;

(4) Manually optimize the MS parameters, declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP). In

addition, manually maximize the MS sensitivity by adjusting source temperature, flow rates of curtain gas, collision gas, source gases 1 and 2 and ionSpray voltage.

(5) Finally adjust interface probe position and capillary position for CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in negative ionization mode.

APPENDIX 6 Information About Validation Study

Instrument Description

HPLC	:	Agilent 1200 SL system
Detector	:	Agilent 6490 Series QQQ

Chromatographic Conditions

Column	:	Atlantis® T3 100 mm x 3.0 mm, 3 µm
Column Oven Temperature	:	40°C
Injection volume	:	80 µL
Stop Time	:	11 minutes
Injection protocol	:	Analyze 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	97	3	0.5
5.2	45	55	0.5
5.6	0	100	0.5
8.5	0	100	0.5
8.6	97	3	0.5
11	97	3	0.5

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.

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

Ion Mode : ES1+Agilent Jet Stream

Polarity : Negative

Capillary : 3000 V

Gas Temperature : 150°C

Gas Flow : 14 L/min

Nebulizer : 45 psi

Sheath Gas Heater : 300

Sheath Gas Flow : 12

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
Confirmatory 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