2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta analytical method entitled "Analytical Method (GRM018.13A) for the Determination of Pymetrozine and its Metabolites CGA215525, CGA300407, CGA294849, CGA371075, CGA363431, CGA359009, CGA363430, CGA255548, CGA249257 and SYN510306 in Water".

This study was designed to satisfy harmonized guideline requirements described in EPA OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods), EC Guidance Documents SANCO/3029/99 Rev.4 (2000), and SANCO/825/00 Rev.8.1 (2010). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

BRIEF SUMMARY OF METHOD

CGA215944, CGA215525, CGA300407, CGA294849, CGA371075, CGA363431, CGA359009, CGA363430, CGA255548, CGA249257, and SYN510306 are analyzed by direct injection of water samples using high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ of the method is 0.05 ppb (µg/L) for each of the analytes.

The determination of CGA215944, CGA215525, CGA363431, CGA294849, CGA371075, CGA363431, CGA359009, CGA363430, CGA255548, CGA249257, and SYN510306 are analyzed by direct injection of water samples using ultra-performance liquid chromatography (UPLC) with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ of the method is 0.05 ppb (µg/L) for each of the analytes.

1. For analysis of CGA294849 and CGA371075, an aliquot was taken from a water sample. The sample was injected for residue analysis using LC-MS/MS after centrifugation.

2. For analysis of CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306, 0.1 mL of 10% NH_4OH solution was added to the 20 mL sample. The sample was injected for residue analysis using LC-MS/MS after centrifugation.

3. For analysis of CGA249257, 10 ml of methanol was added to 10 mL of a water sample and vortexed. The sample was evaporated until aqueous remaining was 1 mL or less under a gentle stream of N_2 in a bath set at a temperature of approximately 50°C. Low recovery, control, and reagent blank ground water samples were diluted to 1 mL with water. Low recovery, control, and reagent blank surface water samples were diluted to 1 mL with 0.05% acetic acid. High recovery ground water samples were diluted to 10 mL with water. High recovery surface water samples were diluted to 2 mL with 0.05% acetic acid. The sample was injected for residue analysis using LC-MS/MS after centrifugation.

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:

ANALYTES	STANDARD	LOT NUMBER	PURITY (%)	EXPIRATION DATE	STORAGE
CGA294849	RS15022	DAH-XXVII-60	99.9	January 31, 2017	Refrigerator
CGA371075	RS15023	DAH-XXXV-16	99.1	April 30, 2016	Refrigerator
pymetrozine (CGA215944)	RS15007	AMS 522/4	99.5	July 31, 2017	< 30°C
CGA215525	RS15019	NV-XXXIV-41	74.2	August 31,2015	Refrigerator
CGA300407	RS15020	DAH-XXXV-61	98.0	October 31, 2015	Freezer
CGA363431	RS15016	MLA-224/2	95.0	September 30,2015	Refrigerator
CGA359009	RS15021	DAH-XXI-90	96.2	August 31, 2015	Refrigerator
CGA363430	RS15017	CDC-XVII-29-1	89.5	January 31, 2017	Refrigerator
CGA255548	RS15024	CDC-XVII-24-2	99.9	January 31, 2017	Refrigerator
SYN510306	RS15018	MES 278/1	90.0	October 31, 2015	Refrigerator
CGA249257	RS15025	NV-XXXIV-29	98.8	January 31, 2017	Refrigerator

The detailed information is listed in Figures 1-11.
Characterization data for the test/reference standards are maintained by Syngenta Crop Protection, LLC. The Certificate of Analysis is included in Appendix 3.

3.2 Test System

The test systems evaluated in this study were Surface Water and Ground Water untreated (control) water samples transferred from Syngenta Crop Protection, LLC and given PASC sample IDs PASC ID 150104-1, 150104-2, 150208-1, and 150208-2. The water types were chosen because they are believed to represent the water samples the method is designed for. These control water samples were characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archives under Syngenta Study Number TK0048240. GLP characterization results are presented in Table 1 and are summarized below:

PASC Sample ID	Water Type	Sample ID
150104-1	Surface Water	RIMV00312-0001 Surface
150208-2	Surface Water	RIMV00115-0001 Surface
150104-2	Ground Water	RIMV00312-0002 Ground
150208-1	Ground Water	RIMV01115-0002 Ground

The samples were received at PASC on February 18, 2015 and April 20, 2015, and were stored in a refrigerator at a nominal temperature of 4°C immediately after arrival. The control samples were checked for contamination prior to use in this study by employing the same extraction and determination as described in Syngenta Method GRM018.13A, and they were found to be free of contamination for all of the analytes.

3.3 Equipment and Reagents

The equipment and reagents used for this study were selected and prepared as outlined in the method. Identical or equivalent equipment and materials may have been used, as permitted by the method.

3.3.1 Equipment

Equipment	Description	Supplier
General lab glassware	General lab glassware	Thermoscientific
General lab plastic ware	General lab plastic ware	Thermoscientific
Autosampler vials	Snap cap, 2 mL size	Thermoscientific
LC-MS/MS system Including HPLC and autosampler units	Acquity UPLC system; AB Sciex 6500 triple quadrupole mass spectrometer with Analyst TM software version 1.6.2	Waters Corporation Applied Biosystems
HPLC column	ACE 5 C18 PFP 100 × 3.0 mm, 5µm particle size Agilent Zorbax SB-Aq 50 × 4.5 mm, 3.5 µm particle size Agilent Pursuit XRs 3 Diphenyl 100 × 4.6 mm	ACE Agilent
Centrifuge Devices	Centrifuge 5415R	Eppendorf
Balance	Mettler Toledo, M72237	Mettler Toledo
Balance	Sartorius, LC220S	Sartorius

3.3.2 Reagents

Reagent	Description	Supplier
Water	In house	Milli-Q Water
Water	HPLC grade	Pharmco
Acetonitrile	HPLC grade	Pharmco
Methanol	HPLC grade	J. T. Baker
Ammonium Hydroxide	ACS grade	Sigma Aldrich
Acetic Acid	ACS grade	J. T. Baker
CGA215944, CGA215525, CGA300407, CGA294849 CGA371075, CGA363431, CGA359009, CGA363430, CGA255548, CGA249257, and SYN510306	GLP certified	Syngenta Crop Protection, LLC P.O Box 18300 Greensboro, NC 27419-8300

3.3.3 Preparation of Reagents

a) 50/50 (v/v) Methanol/Water, prepared by mixing 100 mL HPLC grade methanol with 100 mL Milli-Q Water, degas 15 minutes.

b) 50/50 (v/v) Acetonitrile/Water, prepared by mixing 500 mL HPLC grade acetonitrile with 500 mL Milli-Q Water, degas 15 minutes.

c) Needle Wash (100% Methanol), measured 500 mL of Methanol in a bottle, degas 20 minutes.

d) Mobile Phase B (100% Methanol), measured 1000 mL of Methanol in a bottle, degas 20 minutes.

e) 0.006% NH_4OH Solution in Methanol, prepared by mixing 60 µL of NH_4OH Solution (28%-30% w/v) with 1000 mL of Methanol, degas 20 minutes.

f) 0.006% NH_4OH Solution in Water, prepared by mixing 60 µL of NH_4OH Solution (28%-30% w/v) with 1000 mL of Water, degas 20 minutes.

g) 0.05% Acetic Acid in Water, prepared by mixing 0.5 mL of Acetic Acid with 1000 mL of Water, degas 20 minutes.

h) 5 mM Ammonium Acetate in Water, prepared by weighing 0.1937 g of Ammonium Acetate into 500 mL of Water, degas 20 minutes.

i) 10% Methanol in Water, prepared by mixing 10 mL of Methanol and 90 mL of Water.

j) 10% Methanol in Water, prepared by mixing 50 mL of Methanol and 450 mL of Water, degas 20 minutes.

k) Mobile Phase A (100% Water), measured 1000 mL of Water in a bottle, degas 20 minutes.

3.4 Preparation of Standard Solutions

3.4.1 Stock Standard Solutions

3.4.1.1. CGA255548, CGA300407, and CGA249257
Prepare individual 50 - 100 mg/mL stock solutions. Weigh out accurately, using a five figure balance, into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with acetonitrile.

3.4.1.2. CGA215944, CGA215525, CGA294849, CGA371075, CGA363431, CGA359009, and CGA363430
Prepare individual 50 - 100 mg/mL stock solutions. Weigh out accurately, using a five figure balance, into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with 50/50 (v/v) acetonitrile/water.

3.4.1.3. SYN510306
Prepare individual 50 - 100 mg/mL stock solutions. Weigh out accurately, using a five figure balance, into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with 50/50 (v/v) methanol/water.

Example of Stock Standard Solution Preparation

Compound	Weight (g)	Diluted To (mL)	Concentration (µg/mL)	Standard Solution ID
CGA215944	0.007479	100	74.416	S20150330-1
CGA300407	0.009573	100	92.76	S20150330-2
CGA363431	0.009590	100	83.15	S20150330-3
CGA359009	0.008277	100	79.62	S20150330-4
CGA363430	0.007112	100	63.65	S20150330-5
CGA255548	0.008763	100	87.54	S20150330-6
SYN510306	0.007312	100	65.81	S20150330-7
CGA249257	0.007940	100	78.45	S20150330-8
CGA215525	0.010230	100	75.91	S20150330-9
CGA371075	0.008342	100	82.67	S20150330-10
CGA294849	0.008774	100	87.65	S20150330-11

3.4.2 Fortification Standards

Transfer aliquots (around 1 mL) from each stock solution into a 100 mL volumetric flask and dilute to the mark with acetonitrile to yield a 1 µg/mL combined solution containing CGA215944, CGA300407, CGA359009, CGA363430, CGA215525, CGA363431, CGA294849, CGA371075, CGA255548, CGA249257, and SYN510306. Make serial dilutions from the 1 µg/mL combined solution with water to yield combined fortification standard solutions.

Example of Fortification Standard Solution Preparation

Stock Solutions			Fortification				
Standard Solution ID	Compound	Conc. (µg/mL)	Aliquot Taken	Diluted To (mL)	Solvent	Concentration (µL/mL)	Fortification Solution ID
S20150330-1	CGA215944	74.42	1.344	100	ACN	1	F20150402-1
S20150330-2	CGA300407	92.76	1.078				
S20150330-3	CGA363431	83.15	1.203				
S20150330-4	CGA359009	79.62	1.256				
S20150330-5	CGA363430	63.65	1.571				
S20150330-6	CGA255548	87.54	1.142				
S20150330-7	SYN510306	65.81	1.520				
S20150330-8	CGA249257	78.45	1.275				
S20150330-9	CGA215525	75.91	1.317				
S20150330-10	CGA371075	82.67	1.210				
S20150330-11	CGA294849	87.65	1.141				

3.4.3 Intermediate Standards and Calibration Standards

Intermediate calibration standards were prepared by diluting the 1 µg/mL mixed fortification standard with water. Intermediate calibration standard solutions were prepared at concentrations of 40 ng/mL, 20 ng/mL, 8 ng/mL, 4 ng/mL, 2 ng/mL, and 1 ng/mL for CGA294849, CGA371075, CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306. Intermediate callibration standard and solutions were prepared at concentration of 100 ng/mL, 50 ng/mL, 25 ng/mL, 10 ng/mL, 5 ng/mL, and 2.5 ng/mL for analysis of CGA249257.

For analysis of CGA294849 and CGA371075 in ground and surface water, use the intermediate standards and make 1:40 dilutions with untreated control sample final fraction to yield 1 ng/mL, 0.5 ng/mL, 0.2 ng/mL, 0.1 ng/mL, 0.05 ng/mL, and 0.025 ng/mL matrix-matched standards.

For analysis of CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306 in ground and surface water, use the intermediate standards and make 1:40 dilutions with 20 mL untreated control sample and then add 0.1 mL 10% NH_4OH solutions to yield 1 ng/mL, 0.5 ng/mL, 0.2 ng/mL, 0.1 ng/mL, 0.05 ng/mL, and 0.025 ng/mL matrix-matched standards.

For analysis of CGA249257 in ground water, use the intermediate standards and make 1:10 dilutions with ultrapure water to yield 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1 ng/mL, 0.5 ng/mL, and 0.25 ng/mL standards.

For analysis of CGA249257 in surface water, use the intermediate standards and make 1:10 dilutions with 0.05% acetic acid in untreated control sample to yield 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1 ng/mL, 0.5 ng/mL, and 0.25 ng/mL standards.

Individual calibration curves were generated to quantify the residues of CGA215944, CGA300407, CGA359009, CGA363430, CGA215525, CGA363431, CGA294849, CGA371075, CGA255548, CGA249257, and SYN510306.

Example of Intermediate Calibration Solution Preparation

Stock Solution			Intermediate Calibration Solution				
Standard Solution ID	Compound	Conc. (ng/mL)	Aliquot Taken (µL)	Diluted To (mL)	Solvent	Conc. (ng/mL)	Intermediate Calibration Solution ID
F20150402-1	Mixed: 11 compounds	1000	0.4	10	Water	40	I20150402-1
I20150402-1		40	5	10	Water	20	I20150402-2
I20150402-2		20	4	10	Water	8	I20150402-3
I20150402-3		8	5	10	Water	4	I20150402-4
I20150402-4		4	5	10	Water	2	I20150402-5
I20150402-5		2	5	10	Water	1	I20150402-6

Example of Matrix-Matched Calibration Standard Solution Preparation

Intermediate Calibration Solution			Calibration Solution				
Intermediate Calibration Solution ID	Compound	Conc. (ng/mL)	Aliquot Taken (mL)	Diluted To (mL)	Solvent	Conc. (ng/mL)	Calibration Solution ID
I20150402-1	Mixed: 11 compounds	40	0.125	5	untreated control sample final fraction	1	C20150402-1
I20150402-2		20	0.125	5		0.5	C20150402-2
I20150402-3		8	0.125	5		0.2	C20150402-3
I20150402-4		4	0.125	5		0.1	C20150402-4
I20150402-5		2	0.125	5		0.05	C20150402-5
I20150402-6		1	0.125	5		0.025	C20150402-6

3.5 Analytical Procedures and Modifications

3.5.1 Modifications

Modifications were made to the method with regard to the sample preparation, injection volume, mobile phase gradient, and mass spectrometer parameters. The details are listed in Section 3.5.5.

3.5.2 Sample Preparation and Fortifications

To conduct sample analysis, control water samples were taken from the storage refrigerator and allowed to equilibrate to room temperature prior to use. For the analysis of CGA294849 and CGA371075, a total of 12 samples (10 mL each) were transferred into individual polypropylene centrifuge tubes (50 mL size). For the analysis of CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306, a total of 12 samples (20 mL each) were transferred into individual polypropylene centrifuge tubes (50 mL size). For the analysis of CGA249257, a total of 12 samples (10 mL each) were transferred into individual polypropylene centrifuge tubes (50 mL size). The samples were analyzed as an analytical set: two control samples, five LOQ recovery samples, and five 10X LOQ samples. Appropriate amounts of mixed fortification standard solutions were spiked into the samples to yield the LOQ (0.05 µg/L) and 10X LOQ (0.5 µg/L) concentrations. A calibrated micropipette was used for the fortification. Two samples (untreated water) were not spiked with fortification solutions and were used as control samples. In addition, one reagent blank sample was analyzed along with each analytical set.

Details about the fortifications are summarized as follows:

Matrix	Compounds	Fortification Vol. (mL)	Fortification Conc. (µg/L)	Sample Vol. (mL)	Final Conc. (µg/L)	Replicates
Ground Water Or Surface Water	CGA294849, CGA371075	0.25	2	10	0.05	5
		0.25	20	10	0.5	5
	CGA215944, CGA215525, CGA363431, CGA363431, CGA359009, CGA363430, CGA255548, SYN510306	0.5	2	20	0.05	5
		0.5	20	20	0.05	5
	CGA249257	0.25	2	10	0.05	5
		0.25	20	10	0.5	5

3.5.3 Analysis of CGA294849 and CGA371075

Transfer an aliquot of 1.0 mL of the sample into a 1.5 mL centrifuge tube. Samples were centrifuged at approximately 8000 rpm with refrigeration at 10°C for about 3 minutes. For each of the samples, the supernatant was transferred to a suitable injection vial for LC-MS/MS analysis.

3.5.4 Analysis of CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306

Add 0.1 mL of 10% NH_4OH solution to the 20 mL sample. Cap the tube securely and vortex mix for 30 seconds. Transfer an aliquot of 1.0 mL of the sample into a 1.5 mL centrifuge tube. Samples were centrifuged at approximately 8000 rpm with refrigeration at 10°C for about 3 minutes. For each of the samples, the supernatant was transferred to a suitable injection vial for LC-MS/MS analysis.

3.5.5 Method Modifications

3.5.5.1 Analysis of CGA249257 in Ground Water/Extraction of Analytes

a) Transfer 10 mL of ground water sample into a 50 mL centrifuge tube.
b) Add 10 mL of methanol to the sample and vortex.
c) Evaporate the sample until aqueous remaining is 1 mL or less under a gentle stream of N_2 in a bath set at a temperature of approximately 50°C.
d) Dilute the low recovery samples, control samples, and reagent blank samples to 1 mL with water and the high recovery samples to 10 mL with water.

e) Transfer approximately 1 mL of samples into a 1.5 mL centrifuge tube. The samples were centrifuged at approximately 8000 rpm with refrigeration at 10°C for about 3 minutes.
f) For each of the samples, the supernatant was transferred to a suitable injection vial for LC-MS/MS analysis.
g) The mobile phase gradient was modified to the following:

Time	% A	% B
0	88	12
1	88	12
5.5	50	50
7.0	5	95
10	5	95
10.1	88	12
14	88	12

3.5.5.2 Analysis of CGA249257 in Surface Water/Extraction of Analytes

a) Transfer 10 mL of Surface water sample into a 50 mL centrifuge tube.
b) Add 10 mL of methanol to the sample and vortex.
c) Evaporate the sample until aqueous remaining is 0.5 mL or less under a gentle stream of N_2 in a bath set at a temperature of approximately 50°C.
d) Dilute the low recovery samples, control samples, and reagent blank samples to 1 mL with 0.05% acetic acid in water and the high recovery samples to 2 mL with 0.05% acetic acid in water.
e) Transfer approximately 1 mL of the samples into a 1.5 mL centrifuge tube. The samples were centrifuged at approximately 8000 rpm with refrigeration at 10°C for about 3 minutes.
f) For each of the samples, the supernatant was transferred to a suitable injection vial for LC-MS/MS analysis.

3.5.5.3 Injection Volume

a) An injection volume of 75 µL was used for all analytes.

3.5.5.4 Linearity Criteria

a) A linearity criteria of $r \geq 0.99$ was used for all analytes.

3.5.5.5 Calibration Data Selection

a) Only calibration standards that back-calculated to ± 20% of their respective nominal concentrations were used to generate the calibration curve for all analytes.

3.5.5.6 Recovery Criteria

a) A recovery criteria of 70% - 111% was used for all analytes.

3.5.5.7 Mass Spectrometer Parameters

a) Mass spectrometer parameters were optimized for all analytes.

3.5.5.8 Analysis of CGA294849 and CGA371075 in Ground Water

a) Five samples were run between standards.

3.6 Instrumentation

3.6.1 LC-MS/MS Instrument Description for Analysis of CGA294849 and CGA371075

LC System:	Waters Acquity UPLC system
MS Detector:	Applied Biosystems Sciex API 6500 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2

Chromatographic Conditions

Flow Rate:	0.4 mL/min
Column:	ACE 5 C18 PFP 100×3.0 mm, 5 µm
Injection Vol.:	75 µL
Run Time:	12 minutes
Mobile Phase A:	Water
Mobile Phase B:	MeOH

Isocratic/Gradient Flow:

Time	% A	% B	Flow Rate (mL/min)
0	100	0	0.4
1	100	0	0.4
5.5	50	50	0.4
7.0	5	95	0.4
8.5	5	95	0.4
8.6	100	0	0.4
12	100	0	0.4

Approximate Retention Times of Analytes:

Analyte	Retention Time (min)
CGA294849	3.4
CGA371075	5.2

Mass Spectrometer Conditions

Interface:	TurboIon Spray
Polarity:	Negative
Curtain gas (CUR):	Nitrogen set at 30
Temperature (TEM):	580°C
Ionspray voltage:	-4500V
Collision gas setting (CAD):	Nitrogen set at 8.00 (arbitrary units)
Gas 1 (GS1):	Air set at 65.00
Gas 2 (GS2):	Air set at 55.00
Interface heater (ihe):	On
Scan type:	MRM

Analyte	MS/MS Transition	Dwell (ms)	DP	EP	CE	CXP
CGA294849: ESI Negative						
Primary	141.0→42.0	120	-32	-7	-34	-10
Confirmatory	141.0→124.9	120	-32	-8	-17	-20
CGA371075: ESI Negative						
Quantification	140.1→42.1	120	-33	-9	-38	-11
Confirmatory	140.1→67.8	120	-35	-9	-20	-5

3.6.2 LC-MS/MS Instrument Description for Analysis of CGA215944, CGA215525, CGA300407, CGA363431, CGA359009, CGA363430, CGA255548, and SYN510306

LC System:	Waters Acquity UPLC system
MS Detector:	Applied Biosystems Sciex API 6500 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2

Chromatographic Conditions

Flow Rate:	0.4 mL/min
Column:	Zorbax SB-Aq, 4.5 x 50 mm, 3.5 µm
Injection Vol.:	75 µL
Run Time:	25 minutes
Mobile Phase A:	0.006% NH_4OH in Water
Mobile Phase B:	0.006% NH_4OH in MeOH

Isocratic/Gradient Flow:

Time	% A	% B	Flow Rate (mL/min)
0	100	0	0.4
1	100	0	0.4
5.5	50	50	0.4
7.0	5	95	0.4
15.0	5	95	0.4
15.1	100	0	0.4
25	100	0	0.4

Approximate Retention Times of Analytes:

Analyte	Retention Time (min)
CGA215944	7.4
CGA215525	4.1
CGA300407	6.5
CGA363431	5.8
CGA359009	7.0
CGA363430	5.5
CGA255548	4.6
SYN510306	6.3

Mass Spectrometer Conditions

Interface:	TurboIon Spray
Polarity:	Positive
Curtain gas (CUR):	Nitrogen set at 30
Temperature (TEM):	620°C
Ionspray voltage:	2500V
Collision gas setting (CAD):	Nitrogen set at 8 (arbitrary units)
Gas 1 (GS1):	Air set at 65.00
Gas 2 (GS2):	Air set at 65.00
Interface heater (ihe):	On
Scan type:	MRM

Analyte	MS/MS Transition	Dwell (ms)	DP	EP	CE	CXP
CGA215944: ESI Positive						
Primary	217.8→105.0	50	62	10	27	9
Confirmatory	217.8→78.0	50	62	10	60	10

CGA215525: ESI Positive						
Primary	129.0→45.0	50	60	10	17	7
Confirmatory	129.0→88.0	50	45	10	16	10
CGA300407: ESI Positive						
Primary	108.2→80.2	50	61	8	23	6
Confirmatory	108.2→53.2	50	68	8	33	14
CGA363431: ESI Positive						
Primary	250.1→121.1	50	95	10	26	15
Confirmatory	250.1→103.1	50	95	8	55	15
CGA359009: ESI Positive						
Primary	234.0→105.2	50	50	10	29	10
Confirmatory	234.0→92.1	50	50	10	41	10
CGA363430: ESI Positive						
Primary	248.0→121.1	50	80	10	23	18
Confirmatory	248.0→103.1	50	80	10	45	15
CGA255548: ESI Positive						
Primary	124.0→106.1	50	58	10	23	9
Confirmatory	124.0→78.1	50	58	10	32	10
SYN510306: ESI Positive						
Primary	234.0→121.3	50	55	10	29	8
Confirmatory	234.0→103.1	50	55	5	52	8

3.6.3 LC-MS/MS Instrument Description for Analysis of CGA249257

LC System:	Waters Acquity UPLC system
MS Detector:	Applied Biosystems Sciex API 6500 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2

Chromatographic Conditions

Flow Rate:	0.4 mL/min
Column:	ACE Excel 3 C18 AR, 150 × 3.0 mm, 5 µm
Column Oven Temp:	40°C
Injection Vol.:	75 µL
Run Time:	14 minutes
Mobile Phase A:	0.05% acetic acid in water
Mobile Phase B:	MeOH

Isocratic/Gradient Flow (Ground Water ILV):

Time	% A	% B	Flow Rate (mL/min)
0	88	12	0.4
1	88	12	0.4
5.5	50	50	0.4
7.0	5	95	0.4
10	5	95	0.4
10.1	88	12	0.4
14	88	12	0.4

Isocratic/Gradient Flow (Ground Water Solution Stability, Surface Water ILV and Solution Stability):

Time	% A	% B	Flow Rate (mL/min)
0	95	5	0.4
1	95	5	0.4
5.5	50	50	0.4
7.0	5	95	0.4
10	5	95	0.4
10.1	95	5	0.4
14	95	5	0.4

Approximate Retention Times of Analytes:

Analyte	Retention Time (min) Ground Water ILV	Retention Time (min) Ground Water Solution Stability	Retention Time (min) Surface Water ILV and Solution Stability
CGA249257	3.0	3.7	3.7

Mass Spectrometer Conditions

Interface:	TurboIon Spray
Polarity:	Positive
Curtain gas (CUR):	Nitrogen set at 30
Temperature (TEM):	650°C
Ionspray voltage:	5500
Collision gas setting (CAD):	Nitrogen set at 8 (arbitrary units)
Gas 1 (GS1):	Air set at 50.00
Gas 2 (GS2):	Air set at 60.00
Interface heater (ihe):	On
Scan type:	MRM

Analyte	MS/MS Transition	Dwell (ms)	DP	EP	CE	CXP
CGA249257: ESI Positive						
Primary	114.0→73.1	200	100	3	15	17
Confirmatory	114.1→71.1	200	100	3	17	6

3.7 Data Acquisition

The peak integration and peak area count quantitation were performed by PE Sciex Analyst® (Version 1.6.2). A best-fit linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The correlation coefficient (r) for the calibration curves for each analytical set was greater than or equal to 0.99. Recovery results were computed for each sample.
A statistical treatment of the data includes the calculation of averages, standard deviations, and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package.