1.0 INTRODUCTION

1.1 Purpose of the Study

The objective of this independent laboratory validation (ILV) study was to demonstrate that Analytical Method ADPEN M1408, “The Determination of Residues of CGA-152005 and its Metabolites, CGA-300406, CGA-159902, and CGA150829 in Soil and Water Matrices Using LC-MS/MS”, could be performed successfully with no prior experience with the method.

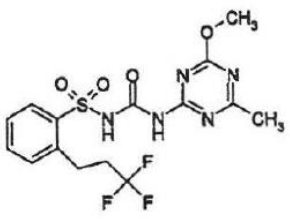
1.2 Summary of the Results

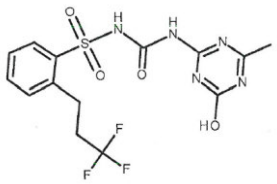
The independent laboratory validation of the analytical method was successfully completed in the first trial.

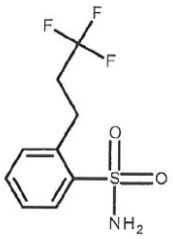
2.0 REFERENCE SUBSTANCE AND SAMPLING HISTORY

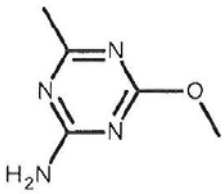
2.1 Reference Materials

Reference standards, prosulfuron (CGA152005) and its metabolites CGA300406, CGA159902, and CGA150829, were used for fortifications and LC-MS/MS calibration. Concentrated (stock), fortification and calibration standards were prepared according to the analytical method. The certificates of analysis for all reference substances are presented in Appendix A. Standard solutions prepared for this study were stored in freezer E-109, which operated at an average temperature of −21 °C. A brief description of the reference standard used in this study is presented below.

Standard Name:	**CGA152005**
Alternate Name:	**Prosulfuron**
CA Index Name:	Benzenesulfonamide, N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino}carbonyl}-2-(3,3,3-trifluoropropyl)-
IUPAC Name:	1-(4-methoxy-6-methyl-biazin-2-yl)-3-[2-(3,3, 3-trifluoropropyl}-phenylsulfonyl]-urea
CAS Number:	94125-34-5
Product Code:	A9417A
Lot Number:	AMS 509/3 (731405)
Molecular Formula:	$C_{15}H_{16}F_3N_5O_4S$
Molecular Weight (g/mol):	419.4 g/mol
Purity:	99.0%
Storage:	Room Temperature
Expiration Date:	End of February 2019
Source:	Syngenta Crop Protection, LLC., Greensboro, NC
Structural Formula:	

Syngenta Code:	CGA300406
Synonyms:	CSAA382278
Batch Number:	NEH-XV-88
Chemical Purity:	96.9%
Storage Conditions:	Refrigerator
Expiration Date:	June 30, 2016
Structural Formula:	

Syngenta Code:	CGA159902
Synonyms:	CSAA148355
Batch Number:	BPM-XII-17
Chemical Purity:	98.3%
Storage Conditions:	Refrigerator
Expiration Date:	June 30, 2016
Structural Formula:	

Syngenta Code:	CGA150829
Synonyms:	CSAA139483
Batch Number:	CDC-III-91
Chemical Purity:	99.3%
Storage Conditions:	Refrigerator
Expiration Date:	June 30, 2016
Structural Formula:	

2.2 Test System

One soil sample and one water sample was sent from R&D Research Farm, Inc. on July 15, 2014 and received by ADPEN Laboratories, Inc. on July 21, 2014. Upon receipt, the samples were stored in refrigerator E-57, which operated at an average temperature of 4 °C during the course of the validation study.

The Laboratory Information Management System (LIMS) provided unique laboratory analysis codes (water = 150527001-001 and soil = 150527001-002) for each control sample, which is cross-referenced on detailed analytical data reports to the sample identification number.

3.0 TECHNICAL PROCEDURE

Analytical Method ADPEN M1408, "The Determination of Residues of CGA-152005 and its Metabolites, CGA-300406, CGA-159902, and CGA150829 in Soil and Water Matrices Using LC-MS/MS" was used for the analysis of water and soil samples.

3.1 Preparation of Solutions

All solutions used for the conduct of the ILV were prepared as described in the analytical method. Below are the solutions and solvent mixtures used for this study and their composition:

Description	Code	Composition
HPLC Mobile Phase A	**LC1**	**0.1% Formic Acid in Water** Add 1 mL of formic acid into a 1 L volumetric flask and bring to the mark with HPLC water. Mix well to ensure complete homogeneous solution.
HPLC Mobile Phase B	**LC2**	**0.1% Formic Acid in Acetonitrile** Add 1 mL of formic acid into a 1 L volumetric flask and bring to the mark with ACN. Mix well to ensure complete homogeneous solution.

3.2 Preparation of Standard Solutions

All stock, fortification, and calibration standard solutions were stored frozen in amber bottles at approximately −24 °C and were brought to room temperature prior to use. Example preparations of standard solutions are presented in Table 18.

3.2.1 Stock Standard Solutions

Stock standard solutions were prepared as per method in 10-mL volumetric flasks. The 1 µg standards were dissolved, using sonication and/or vortexing and then diluted to the mark.

3.2.2 Fortification Standard Solutions

Fortification solutions were prepared at 1, 10, 100, and 1000 ng/mL using the scheme shown in the method. Mixed standard solution for fortifications were prepared by combining individual stock solutions of prosulfuron and its metabolites in volumetric flasks. The two volumetric flasks

containing the fortification standards were diluted volumetrically with acetonitrile as described in the method and complete homogeneous solutions were accomplished by sonication and/or vortexing.

3.2.3 Calibration Standard Solutions

Calibration standard solutions for LC-MS/MS analysis were prepared using stock or intermediate solutions described above and in the method by diluting them as noted in Table 18.

3.3 Water Analysis

Water control samples were allowed to equilibrate to room temperature and inverted several times to ensure homogeneity. A 10-mL aliquot was transferred to a culture tube. Recovery samples were fortified at the LOQ and 10× LOQ, as described in the lab benchsheet, and vortexed thoroughly. Samples were filtered and a portion of the sample was added to an HPLC vial and analyzed by LC-MS/MS.

3.4 Soil Analysis

A 10-g aliquot of soil control samples were transferred to a 50-mL culture tube. Recovery samples were fortified at the LOQ and 10× LOQ, as described in the lab benchsheet. 25-mL of extraction solvent was added to the samples and extracted for approximately one minute using the Omni Bead Ruptor at 4m/s. Samples were centrifuged for 30 minutes at 4150 rpm. A 5-mL aliquot of extract was evaporated at 50 °C to approximately 0.75 mL. The samples were reconstituted to a final volume of 5 mL, shaken, and centrifuged for 30 min at 4150 rpm. Samples were filtered and a portion of the sample was added to an HPLC vial and analyzed by LC-MS/MS.

3.5 LC-MS/MS Instrumentation and Conditions

The LC-MS/MS instrumentation and conditions including the primary and secondary mass transitions (m/z) monitored during this validation study are presented in Table 19.

4.0 LIMITS OF QUANTITATION AND DETECTION

The method limit of quantitation (LOQ) for prosulfuron and its metabolites CGA300406, CGA159902, and CGA150829 is 0.1 ppb in soil and 0.01 ppb in water. The limit of detection (LOD) was considered to be 0.02 ppb in soil and 0.002 ppb in water which is 20% of the LOQ, respectively.

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substance at multiple concentrations.

A standard curve was prepared for each analyte by injecting standard solutions at appropriate concentrations. Calibration standard concentrations ranged from 0.002 to 10 ng/mL. A calibration standard was interspersed with sample injections. Agilent's MassHunter software

created the standard curve based on linear regression using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves are presented in Figure 1. Representative chromatograms of calibration standards for all mass transitions (m/z) for all analytes are presented in Figures 2 through 5.

Peak integration and quantitation were performed within MassHunter; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within the LIMS and reported in Microsoft® Excel spreadsheet as detailed analytical data reports, which are presented in Appendix C.

The following equations are used for residue and recovery calculations for water samples:

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y-b}{m}$

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

b) Amount of sample injected (mL)= $\frac{\text{injection size (mL)}}{\text{final volume (mL)}} \times \text{sample amount (mL)}$

c) Residue found (ppb) = $\frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}}$

d) Recovery (%) = $\frac{\text{Residue in sample (ppb)}}{\text{Amount fortified (ppb)}} \times 100$

As an example, calculations to obtain prosulfuron (primary mass transition, 418.1 → 138.9 *m/z*) recovery results using 15070604-Recovery1-2 from analytical set WO-15070604 are shown below:

a) Calibration curve: y = 154054.220667x - 0.624215

Solving for x: $x = \frac{55 - 0.624215}{154054.220667} = 0.0004$ ng

b) Amount of sample injected (mL) = $\frac{0.04 \text{ mL}}{10 \text{ mL}} \times 10 \text{ mL} = 0.04 \text{ mL}$

c) Residue found (ppb) = $\frac{0.0004 \text{ ng}}{0.04 \text{ mL}} = 0.0100$ ppb

d) Recovery (%) = $\frac{0.0100 \text{ ppb}}{0.01 \text{ ppb}} \times 100 = 100\%$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

13.0 REFERENCES

[1] Tarkalanov, N., Perez, R., Adams, J., Technical Procedure of Method ADPEN M1408: "Method For The Determination of Prosulfuron and Its Metabolites in Paddy Water and Soil by LC-MS/MS", 2015.

Table 1 Flow Diagram of the Technical Procedure

Flowchart for the Analysis of Water Samples

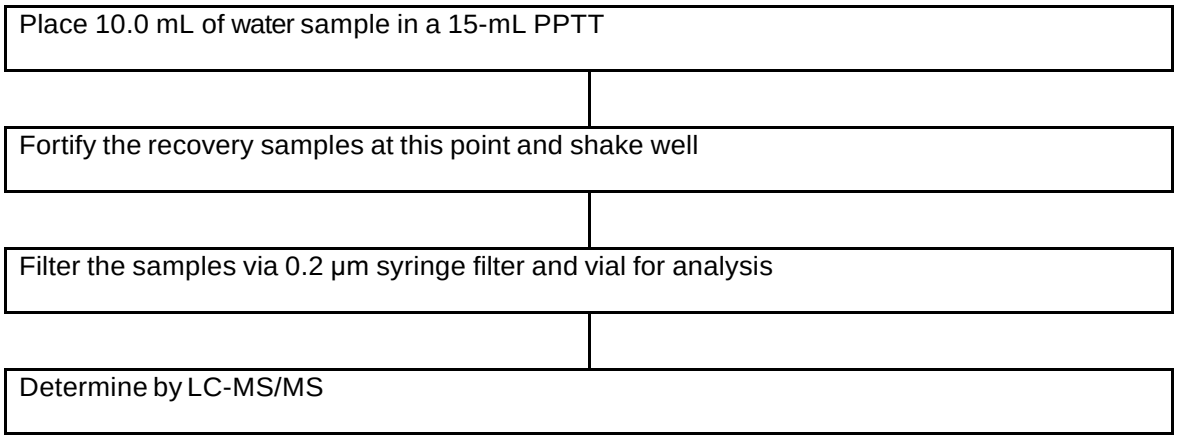


Table 1 Flow Diagram of the Technical Procedure (continued)

Flowchart for the Analysis of Soil Samples

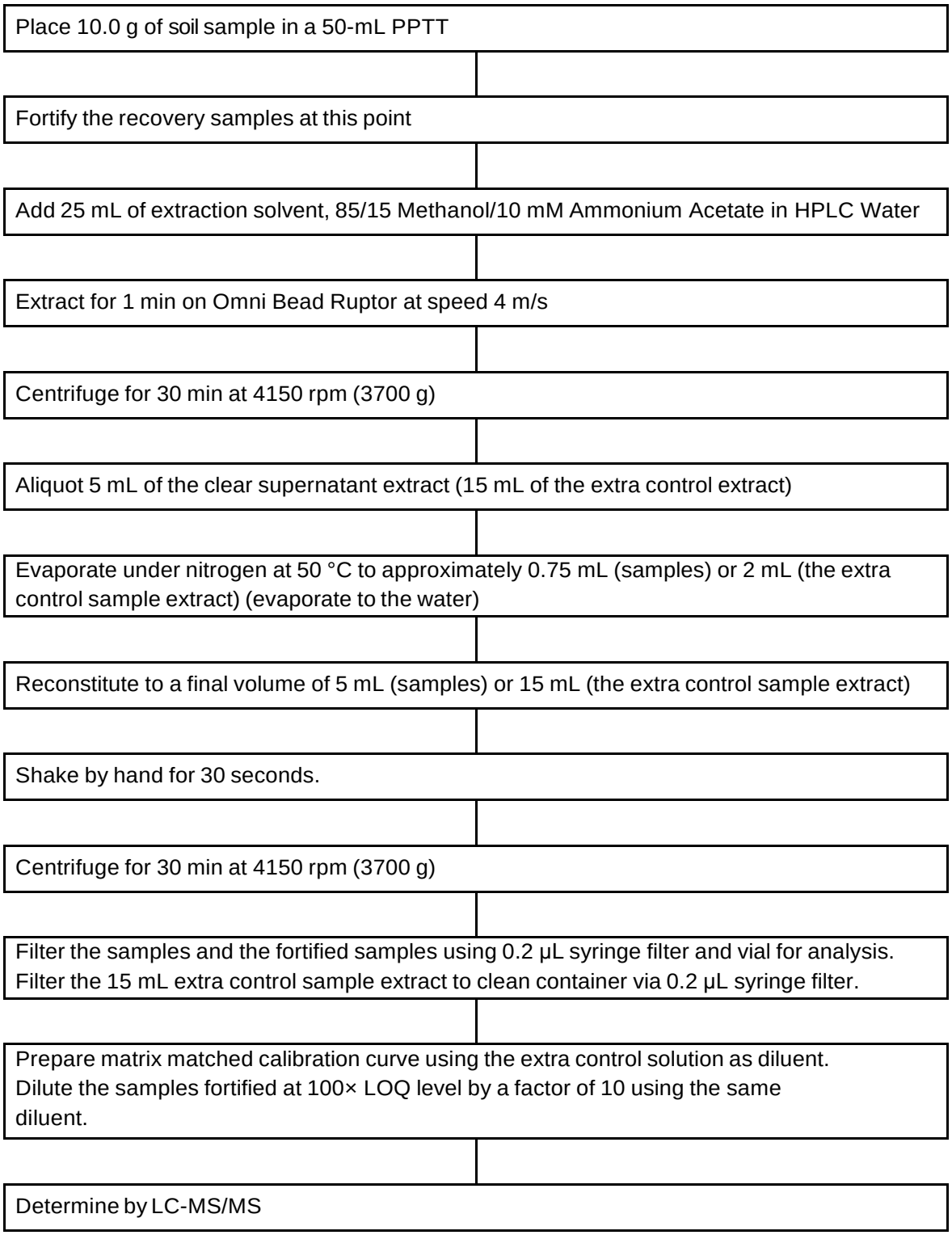


Table 18 Example Standard Solutions Preparation and Dilution Data

Stock Standard Solutions

Conc. Standard	Analyte	Analytical Standard	Amount analyte Weighed (mg)	Final Dil. Vol. (mL)	Final Conc. (mg/mL)	Prep. Date
C7814	Prosulfuron (CGA152005)	P5156	2.01	10	0.19899	5/8/2015
C7815	CGA150829	P5155	2.02	10	0.200586	
C7816	CGA159902	P5154	2.04	10	0.200532	
C7866	CGA300406	P5317	2.06	10	0.19961	

Intermediate Standard Solutions

Int. Standard	Analyte	Parent Conc. Std. No.	Parent Conc. (ng/µL)	Aliquot Vol. (mL)	Dil. Vol. (mL)	Final Conc. (ng/µL)	Prep. Date
I8367	Prosulfuron	C7814	199	0.5	10.0	10.0	5/15/2015
	CGA150829	C7815	200.6				
	CGA159902	C7816	200.5				

Calibration Standard Solutions

Standard Number	Analyte	Standard Code	Standard Conc. (ng/mL)	µL of Standard (100 ng/mL)	µL of Standard (10 ng/mL)	µL of Standard (1 ng/mL)	µL of Standard (0.1 ng/mL)	Solvent Volume (µL)
Cal1	Prosulfuron 4-Mix	W11812-1	10.000	100				900
Cal2		W11812-2	6.000	60				940
Cal3		W11812-3	3.000	30				970
Cal4		W11812-4	1.000		100			900
Cal5		W11812-5	0.500		50			950
Cal6		W11812-6	0.300		30			970
Cal7		W11812-7	0.100			100		900
Cal8		W11812-8	0.050			50		950
Cal9		W11812-9	0.020			20		980
Cal10		W11812-10	0.008				80	920
Cal11		W11812-11	0.005				50	950
Cal12		W11812-12	0.002				20	980

Table 19 Instrument Conditions and Parameters

Chromatographic System	Agilent 1290 UPLC			
Analytical Column	Acquity HSS T3, 2.1 x 150 mm, 1.8 µm (S/N 01663509716061)			
Column Temperature	50 °C			
Injection Volume	20 µL			
Mobile Phase A	0.1% Formic Acid in HPLC Water			
Mobile Phase B	0.1% Formic Acid in ACN			
Flow Rate:	0.6 mL/min			
Gradient	Time (min)	Flow (mL/min)	A (%)	B (%)
	0	0.6	99	1
	0.5	0.6	99	1
	2.4	0.6	40	60
	4.25	0.6	35	65
	4.26	0.6	0	100
	5.25	0.6	0	100
	5.26	0.6	99	1
	5.75	0.6	99	1

Detection System	Agilent 6490 Triple Quad			
Interface:	ElectroSpray Ionization (ESI)			
Gas Flow:	14 L/min			
Temperature:	200 °C			
Capillary (V):	3000			
V Charging:	1500			
Nebulizer (psi):	45			
Sheath gas heater:	300			
Sheath gas flow:	12			
MRM Conditions	**Prosulfuron (CGA152005) (Primary)**	**Prosulfuron (CGA152005) (Secondary)**	**CGA300406 (Primary)**	**CGA300406 (Secondary)**
MS1:	418.08	418.08	404.06	404.06
MS2:	138.90	251.90	124.90	82.00
Dwell time:	100	100	50	50
Frag (V):	380	380	380	380
Collision Energy (V):	20	8	16	56
Cell Acc (V):	7	7	7	7
Polarity:	Negative		Negative	
MRM Conditions	**CGA159902 (Primary)**	**CGA159902 (Secondary)**	**CGA150829 (Primary)**	**CGA150829 (Secondary)**
MS1:	252.03	252.03	141.08	141.08
MS2:	211.90	127.90	57.00	42.10
Dwell time:	100	100	100	200
Frag (V):	380	380	380	380
Collision Energy (V):	16	20	16	28
Cell Acc (V):	7	7	7	7
Polarity:	Negative		Positive	