This study was designed to satisfy harmonized guideline requirements described in OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

This method is used for the determination of CGA-131036, N-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-aminocarbonyl)-2-(2-chloroethoxy)benzenesulfonamide, in soil. This method is a modification of AG-493 where in the Bond Elute CN column clean-up of AG-493 is replaced by the first of two HPLC columns. This modification allows the limit of determination of the method to be decreased to 0.01 ppm as established by the lowest fortification levels.

Soil sample is extracted by shaking with 1:1 methanol: sodium carbonate buffer (pH 9). After filtering, a 5-g aliquot of extract is diluted with water and acidified with phosphoric acid. Residues of CGA-131036 are partitioned into methylene chloride and determined by high performance liquid chromatography (HPLC) using a Lichrosorb - CN column coupled with an analytical Zorbax-ODS column and UV detection at 232 nm.

The following modifications were applied to the method and the E-mail authorization is attached, see Appendix 4.

The method describes that the 1st column should be Lichrosorb – CN, 250×4 mm, ~10µm. However, this column is discontinued. The column Lichrosorb – CN, 250×4.6 mm, 5 µm was used instead. As a result, the retention time of CGA-131036 changed to about 12.5 minutes for the 1st column and about 18 minutes for both columns. The column switch time was set at 11.5-13.5 minutes accordingly.

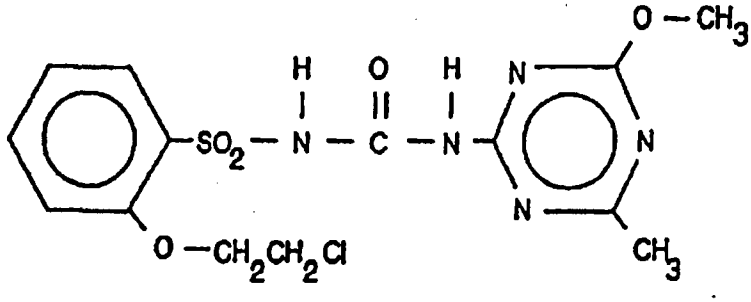
The method Section 6.4.1 states to dissolve the residue in 2.0 ml of 75:25 acetonitrile: water. The 10×LOQ samples were dissolved with 5.0 mL of diluent to obtain a concentration within the curve range.

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 Structure:



Syngenta Code: CGA-131036

Common Name:	Triasulfuron
CAS Name:	Benzenesulfonamide, 2-(2-chloroethoxy)-N-[[4-methoxy-6-methyl-1, 3,5-triazin-2-yl)amino]carbonyl)]-
CAS Number:	82097-50-5
Molecular Weight:	401.8
Standard Reference:	606233
Storage Conditions:	<30°
Purity:	95.4%
Expiration Date:	06/31/2017

Characterization data for the test/reference standard are maintained by the Sponsor, "Syngenta Crop Protection, LLC". The Certificate of Analysis is included in Appendix 2.

The test/reference substance (analytical standard) used in this study was provided by the Sponsor and stored as directed on "Analytical Standards Chain of Custody" documents. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was soil. Control sample(s) used in this study were provided by the Sponsor. Control soil sample(s) were characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0120986.

The untreated control soil samples were received on 08/06/2013 and stored in the freezer (-20°C) at Primera Analytical Solutions Corp. (PASC)

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 AG509 "Analytical Method for the Determination of CGA-131036 by column switching high performance liquid chromatography".

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

3.2.1 Equipment

a) Analytical Balance: Mettler Toledo, Model XS105 (LETS# 129, Calibration Due: 05/2014, weight verification daily)

b) pH Meter: ORION, Model 520A (LETS# 92, Calibration Due: 04/2014, pH calibration daily)

c) Agilent 1100 HPLC equipped with UV detector (LETS# 119, Calibration Due: 02/2014)

d) Column #1: Phenomenex, Lichrosorb CN, 250 x 4.6 mm, 5 µm, S/N: 214155-1

e) Column #2: Waters Symmetry C18, 250 x 4.6 mm, 5 µm, S/N: USBF002633

f) Pipettes: Eppendorf, LETS# 216, Calibration Due: 02/2014, 20-200 µL
Eppendorf, LETS#84-4, Calibration Due: 03/2014, 100-1000µL

g) Agilent ChemStation/ChemStore

3.2.2 Reagents

Name	Manufacture	Lot No.	Expiry Date
Acetonitrile	Pharmco	PB005715ACN-WR-HPLC-0121	09/25/18
Methanol	Pharmco	C1309206	10/03/2018
Methylene chloride	Fluka Analytical	SZBB1520V	01/18/17
Phosphoric acid (85%)	EMD	53100328	09/27/18
Potassium dihydrogen phosphate	EMD	20120111101	09/27/18
Sodium bicarbonate	Sigma-Aldrich	SLBF3956V	07/12/18
Sodium carbonate	Sigma-Aldrich	SLBF4236V	03/25/18
Tetrabutylammonium bromide	Sigma-Aldrich	MKBG5872V	10/02/18
Water	Milli-Q System	N/A	Daily

3.2.3 Preparation of Reagents

Reagents were prepared and stored as recommended in the method and conducted as shown in the following ratio.

Diluent:
Acetonitrile: Water (25:75)

Mobile Phase for column #1:
0.5% Tetrabutylammonium bromide in 25% acetonitrile: 75% 0.001M phosphoric acid

Mobile Phase for column #2:
0.5% Tetrabutylammonium bromide in 30% acetonitrile: 70% pH:7 phosphate buffer

3.3 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in the method and conducted as shown in the following tables.

3.3.1 *Stock Standard*

Stock Standard Solutions (Solvent: Acetonitrile)					
Analytical Standard	**Stock standard No.**	**Amount Weighed (mg)**	**Final Dilution Vol. (mL)**	**Final Conc. (mg/mL)**	**Prep. Date**
CGA-131036	S10181301	100.12	100	0.9551	10/18/2013

3.3.2 Fortification Standard

Stock Solution			Fortification Solution				
Standard Solution ID	**Compound**	**Conc (µg/mL)**	**Aliquot Taken (mL)**	**Diluted to (mL)**	**Solvent**	**Conc (µg /mL)**	**Fortification Solution ID**
S10181301	CGA131036	955.14	2.618	50	ACN	50.01	F10181301
F10181301	CGA131036	50.01	2	50	ACN	2.0	F10181302

3.3.3 Calibration Standard

Stock Solution			Calibration Solution				
Standard Solution ID	**Compound**	**Conc (µg/mL)**	**Aliquot Taken (mL)**	**Diluted to (mL)**	**Solvent**	**Conc (µg/mL)**	**Calibration Solution ID**
S10181301	CGA-131036	955.14	2.618	50	ACN	50.01	C10181301
S10181301	CGA-131036	50.01	1.0	100	ACN	0.500	C10181302
S10181302	CGA-131036	0.500	2.5	100	ACN:Water (25:75)	0.0125	C10181303
S10181302	CGA-131036	0.500	4	100	ACN:Water (25:75)	0.020	C10181304
S10181302	CGA-131036	0.500	2.5	50	ACN:Water (25:75)	0.025	C10181305
S10181302	CGA-131036	0.500	5	50	ACN:Water (25:75)	0.050	C10181306
S10181302	CGA-131036	0.500	10	50	ACN:Water (25:75)	0.100	C10181307
S10181302	CGA-131036	0.500	5	20	ACN:Water (25:75)	0.125	C10181308
S10181302	CGA-131036	0.500	10	20	ACN:Water (25:75)	0.250	C10181309

3.4 Analytical Procedures and Modifications

Analytical Method AG-509 was independently validated as written.

3.4.1 Modifications

Syngenta Method AG-509 was followed as written with two exceptions, see exceptions below.

The method describes that the 1st column should be Lichrosorb – CN, 250×4 mm, ~10µm. However, this column is discontinued. The column Lichrosorb – CN, 250×4.6 mm, 5 µm was used instead. As a result, the retention time of CGA-131036 changed to about 12.5 minutes for the 1st column and about 18 minutes for both columns. The column switch time was set at 11.5-13.5 minutes accordingly.

The method Section 6.4.1 states to dissolve the residue in 2.0 mL of 75:25 acetonitrile: water. The 10×LOQ samples were dissolved with 5.0 ml of diluent to obtain a concentration within the curve range.

3.4.2 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard to LOQ and 10X LOQ concentrations as per method.
Fortifications used in this method validation are as follows:

Matrix	Fortification Vol. (µL)	Fortification Conc. (µg/mL)	Sample Wt. (g)	Final Conc. (mg/kg)	Replicates
Untreated control soil+ LOQ	100	2	20	0.01	5
Untreated control soil+ 10×LOQ	40	50	20	0.1	5

3.4.3 Extraction Procedure

As indicated by method AG-509, the following extraction steps were performed:

1. Weigh a 20-gram subsample from a well-homogenized stone-free soil sample into a 16-oz. square amber jar. Add 200 mL of 1:1 methanol: carbonate buffer and cap the jar using plastic liner to prevent solvent losses during shaking.
2. Extract the soil sample by shaking on a mechanical shaker for two hours.
3. Filter the extracted sample through a filter consisting of a ball of glass wool inside a Reeve Angel filter paper, inside a Whatman 2V filter paper, into an 8-oz. Boston round bottle.
4. Transfer a 5-g aliquot (~51 mL, volume corrected for moisture content of soil) of the extract from Section 3 into a 250-mL separatory funnel.

5. Add 100 mL of water and 10mL of 1.2M phosphoric acid. Check the pH of the solution with pH paper. Adjust the pH to 4 by addition of 1.2M phosphoric acid, if necessary.

6. Add 25 mL of methylene chloride to the separatory funnel and shake vigorously for 30 seconds. Allow the layers to separate, then drain off the lower, methylene chloride layer into a 100-mL round-bottom flask.

7. Repeat Section 5 and combine the methylene chloride fractions in the 100-mL round bottom flask.

8. Evaporate the contents of the round bottom flask to approximately 5 to 10 mL and quantitatively transfer the sample to a 50-mL round bottom flask. (The 50-mL round bottom flask is used in order to reduce the surface area available when dissolving the residue before HPLC analysis). Re-evaporate the sample to dryness at 40°C. If any moisture remains after evaporation, add 1-2 mL of acetonitrile to the flask and re-evaporate.

3.5 Instrumentation

HPLC System:	Agilent 1100 Series
Detector:	DAD
Flow Rate:	2.0 mL/min (1.0 m/min for each channel)
Column:	#1 Lichrosorb-CN, 250x4 mm, 5 µm #2 Zorbax-ODS, 4.6x250 mm, 5 µm
Column Oven Temp:	Ambient
Injection Volume:	200µL
Run Time:	24 minute
Retention Time:	about 18 minute
Mobile Phase A:	0.5% Tetrabutylammonium bromide in 25% acetonitrile:75% 0.001M phosphoric acid
Mobile Phase B:	0.5% Tetrabutylammonium bromide in 30% acetonitrile:70% pH:7 phosphate buffer

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by "Agilent Chemstation". A linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recoveries were computed for fortified samples.

A statistical treatment of the data includes the calculation of averages, standard deviations, relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package.