2.0 INTRODUCTION

Described in this report is the independent laboratory validation of Syngenta Analytical Method GRM035.03A (Reference 1) as performed by Syngenta Crop Protection, LLC. This validation was conducted to satisfy EPA GDCI-128969-1196 requesting compound specific information (Reference 3), therefore, CGA150829 was not included in the study objective.

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

The residue analytical method is deemed suitable for the determination of triasulfuron (CGA136036) in ground water.

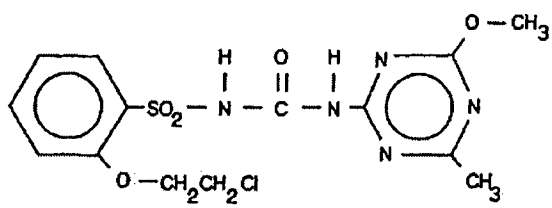
A 25 mL sample of acidified water is loaded onto an Oasis MCX solid phase extraction (SPE) cartridge on which triasulfuron residues are retained. Triasulfuron is eluted from the SPE column with methanol:35% ammonium hydroxide (95/5 v/v). The column eluate is evaporated to @ 0.5 mL then diluted with ultra-pure water. Final determination is performed using LC-MS/MS.

The validated limit of quantitation of method GRM035.03A is 0.01 µg/L (ppb).

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

The test/reference substance was obtained from Syngenta Crop Protection, LLC. The following test/reference substance was used:

Compound Structure	
Syngenta Code:	CGA131036
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° C
Purity:	95.4%
Expiration Date:	6/2017

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

The test/reference substance (Triasulfuron) used in this study was procured from Syngenta Crop Protection, LLC located at the Greensboro facility. All solutions made from triasulfuron standard were stored according to Section 2 of the method.

3.2 Test System

The test system evaluated for this ILV was ground water. This matrix was chosen because it is representative of the matrices the method was designed for and will fulfil EPA information request GDCI-128969-1196. Control samples used in this study were provided by Syngenta Crop Protection, LLC. This control water sample was characterized by AGVISE Laboratories of Northwood, North Dakota and originally reported to Syngenta Archive under Syngenta Study Number TK0048240. GLP characterization results in more detail are presented in Table 1 and summarized below:

Sample ID	pH	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Hardness CaCO3 (mg/L)	SAR	TDS (ppm)
Ground	7.5	16	4.5	6.4	59	0.36	122

3.3 Equipment and Reagents

The equipment and reagents used for the ILV were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents

3.4 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in Section 2 of the method.

3.4.1 Stock Standard

A 100 µg/mL stock solution for triasulfuron was prepared in acetonitrile.

3.4.2 Fortification Standard

Sample fortification solutions containing triasulfuron were prepared by serial dilution in ultra-pure water:acetonitrile (80/20 v/v) from the stock solution. The following solutions were prepared: 10 µg/L, 1.0 µg/L for fortification purposes.

3.4.3 Calibration Standard

Daily use calibration standards were prepared by serially diluting stock and fortification standards using ultra-pure water:acetonitrile (80/20 v/v). For example, to prepare a 0.10 µg/L calibration standard, transfer 0.1 mL of a 1.0 µg/L triasulfuron standard to a 2 mL LC vial and add 0.9 mL ultra-pure water:acetonitrile (80/20 v/v). Using equivalent LC-MS/MS instrumentation described in the method, the following concentration range of standards were prepared and used to construct the calibration plots for primary and confirmatory transitions (0.05 µg/L -10.0 µg/L).

3.5 Analytical Procedures and Modifications

Analytical Method GRM035.03A was successfully independently laboratory validated (ILV) as written using the procedures and instrumentation as recommended by the method.

3.5.1 Modifications

Syngenta Method GRM035.03A was followed as written with the exception of using a 40 µL injection volume instead of 100 µL found in Section 4.2 of the method. An ABSCIEX API 5500 QTRAP LC-MS/MS was used for determination of residue and provided increased sensitivity in comparison to the ABSCIEX API4000 instrumentation that was used to develop the method. This does not affect the quality or integrity of the data.

3.5.2 Fortifications

Untreated control ground water samples were fortified using 250 µL of known amounts of triasulfuron to LOQ and 10X LOQ concentration levels as per method. See Table 2 for detailed fortification levels. Fortifications used in this ILV are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/L)	Final Volume (mL)	Replicates
Ground LOQ	250	1.0	25	5
Ground 10X LOQ	250	10	25	5

3.5.3 Method Summary

As per Analytical Method GRM035.03A a 25 mL sample of acidified water is loaded onto an Oasis MCX solid phase extraction (SPE) cartridge on which triasulfuron residues are retained. Triasulfuron is eluted from the SPE column with methanol:35% ammonium hydroxide (95/5 v/v). The column eluate is evaporated to 0.5 mL then diluted with ultra-pure water to a final volume of 2 mL. Final determination is performed using LC-MS/MS.

3.5.4 Limit of Detection and Limit of Quantitation

The limit of detection (LOD) of the method is defined as the lowest analyte amount injected on column detectable above the mean amplitude of the background noise at the

corresponding retention time. Using the instrumentation described in Section 4 of the method and the sensitivity of the Applied Biosystems API 5500 QTRAP, the limit of detection for analytical method GRM035.03A was estimated as 0.5 pg injected on column. Note that the LOD may vary between runs and from instrument to instrument.

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated. A LOQ of 0.01 µg/L (ppb) in ground water was successfully validated in this study.

3.5.5 Detector Linearity

The linearity of the detector response was assessed using a calibration curve generated with each analysis sequence injected. It was shown that the LC-MS/MS detector response for triasulfuron has a correlation coefficient greater than 0.99 in the range from 0.05 pg/µL to 10.0 pg/µL or 2 pg to 400 pg on column when using a 40 µL injection volume.

Representative plots of the detector responses versus the analyte concentration for all calibration points are presented in the Figures Section.

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by "Analyst Software version 1.5.1". 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 square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

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 Microsoft Office Excel package (2010).

3.7 Matrix Effects

Matrix effects were not observed in the water type tested during the independent laboratory validation and non-matrix matched standards were used. Matrix effects are discussed in Section 8.2 of Analytical Method GRM035.03A.

3.8 Final Extract Stability

Final extract stability for triasulfuron is discussed in Section 8.6 of Analytical Method GRM035.03A.