Test Material:	Triasulfuron
MRID:	49323901
Title:	Triasulfuron, Triasulfuron - Analytical Method for the Determination of Residues of Triasulfuron and its Metabolite CGA150829 in Water. Final Determination by LC-MS/MS, Analytical Method
MRID:	49323902
Title:	Triasulfuron, Triasulfuron - Independent Laboratory Validation of Analytical Method (GRM035.03A) for the Determination of Residues of Triasulfuron in Water. Final Determination by LC-MS/MS, Final Report
EPA PC Code:	128969
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 10/1/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 10/1/14

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 10/1/14

Analytical method for triasulfuron and its product CGA150829 in water

Reports: ECM: EPA MRID No.: 49323901. Oppilliart, S. 2010. Triasulfuron, Triasulfuron - Analytical Method for the Determination of Residues of Triasulfuron and its Metabolite CGA150829 in Water. Final Determination by LC-MS/MS, Analytical Method. Eurofins|ADME Bioanalyses Report No.: GRM035.03A. Syngenta Task No.: TK0005001. Report prepared by Eurofins|ADME Bioanalyses, Vergèze, France, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 107 pages. Final report issued February 23, 2010. ILV: EPA MRID No. 49323902. Mayer, L. and M. Manuli. 2014. Triasulfuron, Triasulfuron - Independent Laboratory Validation of Analytical Method (GRM035.03A) for the Determination of Residues of Triasulfuron in Water. Final Determination by LC-MS/MS, Final Report. Syngenta Report and Task No.: TK0120985. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 154 pages. Final report issued January 22, 2014.

Document No.: MRIDs 49323901 & 49323902

Guideline: 850.6100, 860.1340 (p. 13 of MRID 49323901; p. 9 of MRID 49323902)
OECD ENV/JM/MONO(2007)17
EC SANCO/3029/99 rev. 4 & SANCO/825/00 rev. 7

Statements: ECM: The study was not conducted under either USEPA or OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49323901). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity Certification statements were not provided. ILV: The study was conducted in accordance with USEPA GLP standards (p. 3 of MRID 49323902). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity Certification statement was not provided.

Classification: This analytical method is classified as **Supplemental**. The ILV did not validate the method for triasulfuron in surface water and drinking water matrices, and the registrant did not specify that the ground water used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM. The ILV did not validate the method for triasulfuron product CGA150829. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. LODs differed in the ECM and ILV.

PC Code: 128969

Reviewer: Christopher M. Koper 23 JAN 2015

Christopher M. Koper, M.S., Chemist **Date:** January 23, 2015

Page citations refer to the page numbers located in the bottommost right corner of MRID 49323902 (ILV, since this report contains the ECM), unless noted otherwise.

Executive Summary

This analytical method, Syngenta GRM035.03A, is designed for the quantitative determination of triasulfuron and its product CGA150829 in ground water, surface water and drinking water using HPLC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.01 µg/L. The LOQ is less than the lowest toxicological level of concern (acute and chronic fish and invertebrate data > 50,000 µg/L) in water. The independent laboratory validated the method for analysis of triasulfuron in ground water after one trial. LODs for triasulfuron were set at 0.133-0.222 ng/L in the ECM (dependent upon matrix and quantitation/confirmation method) and 12.5 ng/L in the ILV. The ILV did not validate the method for triasulfuron in surface water and drinking water matrices, and the registrant did not specify that the ground water used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM. The ILV did not validate the method for triasulfuron product CGA150829 in any water matrix.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix[1]	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Triasulfuron	49323901	49323902		Water	23/02/2010	Syngenta	HPLC/MS/MS	0.01 µg/L
CGA150829								

1 The ECM validated the method for both analytes using ground water, surface water, and drinking water. The ILV did not validate the method for analysis of triasulfuron in surface water and drinking water, or for its product CGA150829 in any water matrix.

I. Principle of the Method

Samples (25 mL) of water were acidified with concentrated orthophosphoric acid (50 µL), then loaded onto a Waters Oasis MCX solid phase extraction (SPE) cartridge (60 mg/3 mL) pre-conditioned with methanol and ultra pure water (UPW; Appendix 1, pp. 56-57, 148). The sample container was rinsed with 1% orthophosphoric acid and the rinsate applied to the loaded cartridge. Residues were eluted with methanol:35% ammonia solution (95:5, v:v; 3 mL). The eluate was concentrated to 0.5 mL under nitrogen at 40°C to remove solvent, then brought to 2 mL with UPW and sonicated for analysis using an Applied Biosystems API 4000 LC/MS/MS (Appendix 1, p. 59).

Samples were analyzed for triasulfuron and its product CGA150829 by HPLC (Agilent Zorbax SB-C8, 3 mm x 150 mm, 3.5 µm column, 40 ± 5°C) using a mobile phase of (A) methanol:acetonitrile (50:50, v:v) and (B) 0.2% acetic acid in UPW [percent A:B at 0.0 min. 20:80, 2.0-4.0 min. 50:50, 4.1-6.0 min. 20:80] with MS/MS-ESI (electrospray ionization [TurboIonSpray], positive ion mode) detection and multiple reaction monitoring (MRM; Appendix 1, pp. 59-61, 141). Injection volume was 100 µL. Triasulfuron was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). CGA150829 was identified using one ion transition. Ion transitions monitored were as follows: *m/z* 402.1→167.3 (Q) and *m/z* 402.1→141.2 (C) for triasulfuron and *m/z* 141.1→58.1 for CGA150829. As a confirmatory method for CGA150829, an Agilent Zorbax SB-CN (4.6 mm x 150 mm, 5 µm) HPLC column was substituted (Appendix 1, pp. 60-61, 141).

The ILV performed the method as written with the exceptions of using an AB SCIEX API 5500 QTRAP LC/MS/MS, a 40 µL injection volume, and product GCA150829 was not included in the analysis (pp. 10, 12). The instrument parameter modifications are not considered substantial changes to the ECM.

In the ECM, the LOQ for triasulfuron and CGA150829 was 0.01 µg/L; LODs were estimated at 0.133-0.222 ng/L for triasulfuron and 0.541-2.148 ng/L for CGA150829, dependent upon matrix and quantitation/confirmation method used (Appendix 1, pp. 66, 70). In the ILV, the LOQ for triasulfuron was the same as in the ECM (0.01 µg/L); the LOD was estimated at 0.5 pg (0.0125 pg/µL = µg/L = 12.5 ng/L, based on a 40 µL injection; pp. 12-13).

II. Recovery Findings

ECM (MRID 49323901 & Appendix 1, pp. 45-148 of 49323902): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of triasulfuron and its product CGA150829 in ground water, surface water, and drinking water at fortification levels of 0.01 µg/L (LOQ), and 0.10 µg/L (10x LOQ; Appendix 1, pp. 71-72). Triasulfuron was identified and quantified using two ion transitions. CGA150829 was identified and quantified using differing LC conditions. Matrix-matched calibration standards for both analytes were used for ground water analyses, but not for surface water and drinking water analyses (Appendix 1, pp. 65, 76). The water matrices were characterized (pH, silt content, dissolved organic carbon, total hardness), but the primary source of each matrix was not reported (Appendix 1, p. 70).

ILV (MRID 49323902): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of triasulfuron in ground water at fortification levels of 0.01 µg/L (LOQ) and 0.10 µg/L (10x LOQ; p. 14). Triasulfuron was identified and quantified using two ion transitions. Matrix-matched standards were not used (pp. 9, 13). The method was validated for triasulfuron in ground water at both fortification levels after one trial, with minor instrument parameter modifications (pp. 12, 14). The water matrix was characterized, but the primary source of the matrix was not reported (p. 11; Table 1, p. 18). The ILV did not validate the method for analysis of triasulfuron in surface water and drinking water, or for its product CGA150829 in any water matrix.

Table 2. Initial Validation Method Recoveries for Triasulfuron and Its Product CGA150829 in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water					
	Quantitation method					
Triasulfuron	0.01 (LOQ)	5	80-83	81	1	2
	0.10	5	77-80	79	1	2
CGA150829	0.01 (LOQ)	5	77-86	82	4	5
	0.10	5	88-91	89	1	1
	Confirmation method					
Triasulfuron	0.01 (LOQ)	5	83-88	84	2	3
	0.10	5	84-89	86	2	3
CGA150829	0.01 (LOQ)	5	88-99	92	5	6
	0.10	5	100-104	102	1	1
	Surface Water					
	Quantitation method					
Triasulfuron	0.01 (LOQ)	5	89-96	92	3	3
	0.10	5	94-98	96	2	2
CGA150829	0.01 (LOQ)	5	83-98	90	6	7
	0.10	5	96-100	98	2	2
	Confirmation method					
Triasulfuron	0.01 (LOQ)	5	83-95	90	5	5
	0.10	5	95-99	96	2	2
CGA150829	0.01 (LOQ)	5	81-96	89	7	8
	0.10	5	96-99	98	1	1
	Drinking Water					
	Quantitation method					
Triasulfuron	0.01 (LOQ)	5	90-96	92	2	3
	0.10	5	90-94	92	2	2
CGA150829	0.01 (LOQ)	5	84-102	92	7	8
	0.10	5	93-98	96	2	2
	Confirmation method					
Triasulfuron	0.01 (LOQ)	5	85-92	89	3	3
	0.10	5	92-95	94	1	1
CGA150829	0.01 (LOQ)	5	88-96	92	4	4
	0.10	5	96-99	97	1	1

Data were obtained from Tables 4-7, pp. 30-31 of MRID 49323901 and DER Attachment 2 (standard deviations). Although not specified, example calculations indicate recovery results were uncorrected (pp. 21-23 of MRID 49323901).

1 The three water matrices were partially characterized (pH, silt content, dissolved organic carbon, total hardness); primary sources of the matrices were not reported (Table 1, p. 29 of MRID 49323901).

Table 3. Independent Validation Method Recoveries for Triasulfuron in Ground Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Triasulfuron	Quantitation method					
	0.01 (LOQ)	5	100-110	104	5	5
	0.10	5	91-117	104	10	9
	Confirmation method					
	0.01 (LOQ)	5	94-105	98	5	5
	0.10	5	89-114	102	9	9

Data were obtained from Tables 3-4, pp. 20-21 of MRID 49323902. Although not specified, residue data sheets indicate recovery results were uncorrected (Appendix 3, pp. 153-154).

1 Ground water was characterized by Agvise Laboratories, Northwood, North Dakota (p. 11; Table 1, p. 18); primary source of the matrix was not reported.

III. Method Characteristics

In the ECM, the LOQ for triasulfuron and its product CGA150829 in water was 0.01 µg/L (Appendix 1, p. 66). In the ILV, the LOQ for triasulfuron was the same as in the ECM (pp. 12-13); CGA150829 was not included in the ILV. The LOQ was defined as the lowest analyte concentration in a sample at which the method has been successfully validated (mean 70-110%, RSD ≤20%). In the ECM (API 4000 LC/MS/MS), LODs were estimated at 0.133-0.222 ng/L for triasulfuron and 0.541-2.148 ng/L for CGA150829, dependent upon matrix and quantitation/confirmation method used (Appendix 1, pp. 66, 70). The ECM defined the LOD as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time of the analyte, and estimated the LODs as 3x background noise. In the ILV (API 5000 LC/MS/MS), the LOD for triasulfuron was estimated at 0.5 pg (0.0125 pg/µL = µg/L = 12.5 ng/L, based on a 40 µL injection).

Table 4. Method Characteristics for Triasulfuron and Its Product CGA150829 in Water

	Triasulfuron	CGA150829
Limit of Quantitation (LOQ)	ECM: 0.01 µg/L in ground, surface and drinking water. ILV: 0.01 µg/L in ground water; other matrices were not tested.	ECM: 0.01 µg/L in ground, surface and drinking water. ILV: Not performed.
Limit of Detection (LOD)	ECM: 0.133-0.222 ng/L[1] ILV: 0.5 pg (12.5 ng/L) in ground water.	ECM: 0.541-2.148 ng/L[1] ILV: Not performed.
Linearity (calibration curve r^2 and concentration range)	ECM: r^2 = 0.9986-0.9999 (0.05-2.0 µg/L)[2] ILV: r^2 = 0.9981-0.9985 (0.05-10.0 µg/L)[3]	ECM: r^2 = 0.9983-0.9996 (0.05-2.0 µg/L)[2] ILV: Not performed.
Repeatable	ECM: Yes for ground, surface and drinking water.	
	ILV: Yes for ground water; other matrices were not tested.	ILV: Not performed.
Reproducible	Yes for ground water; other matrices were not tested by ILV.	Not tested by ILV.
Specific	ECM: Yes for ground, surface and drinking water; matrix-matched standards were required for ground water.	
	ILV: Yes for ground water; other matrices were not tested.	ILV: Not performed.

Data were obtained from pp. 12-13; Appendix 1, pp. 58, 65-66, 70, 131-138; Appendix 3, pp. 153-154.

1 Dependent upon matrix and quantitation/confirmation method used (Appendix 1, p. 70).

2 Linearity of the ECM calibration curves was verified by the reviewer (DER Attachment 2).

3 Reviewer-generated r^2 values using results from residue data sheets (DER Attachment 2); copies of provided calibration curves were illegible (Figure 12, p. 28; Figure 24, p. 34).

IV. Method Deficiencies and Reviewer's Comments

1. The ILV did not validate the method for triasulfuron in surface water and drinking water matrices, and the registrant did not specify that the ground water used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM. Matrix-matched calibration standards were required for the ground water matrix used in the ECM validation, but not the ILV (pp. 9, 13; Appendix 1, pp. 58, 65, 76).

2. The ILV did not validate the method for triasulfuron product CGA150829 in any water matrix. Syngenta excluded CGA150829 from the ILV based on the interpretation, by the registrant, that USEPA GDCI-128969-1196 (14-Feb-2013) requesting environmental chemistry methods and laboratory validation in soil and water for guideline 835.6100 Terrestrial Field Dissipation was parent compound (triasulfuron) specific (p. 10). CGA150829 was found to be a major (≥10% of applied) transformation product of triasulfuron in aerobic soil metabolism (MRIDs 49126701, 49126702), aerobic aquatic metabolism (MRID 49126705), and anaerobic aquatic metabolism (MRID 49126706) studies.

3. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. In the ECM, the LOQ for triasulfuron and its product CGA150829 in water was 0.01 µg/L (Appendix 1, p. 66). In the ILV, the LOQ for triasulfuron was the same as in the ECM (pp. 12-13); CGA150829 was not tested in the ILV. The LOQ was defined as the lowest analyte concentration in a sample at which the method has been successfully validated (mean 70-110%, RSD ≤20%). The LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time of the analyte (pp. 12-13; Appendix 1, p. 66). In the ECM (API 4000 LC/MS/MS), LODs were estimated at 3x background noise; 0.133-0.222 ng/L for triasulfuron and 0.541-2.148 ng/L for CGA150829, dependent upon matrix and quantitation/confirmation method used (Appendix 1, p. 70). In the ILV (API 5000 LC/MS/MS), the LOD for triasulfuron was estimated at 0.5 pg (0.0125 pg/µL = µg/L = 12.5 ng/L, based on a 40 µL injection; pp. 12-13).

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

4. The originating laboratory Eurofins|ADME Bioanalyses developed this method (Syngenta Analytical Method GRM035.03A) based on methodology described in Eurofins|ADME Bioanalyses Report S09-03244, Syngenta Method RAM 469/01 and Syngenta Method RAM 490/01 (p. 10; Appendix 1, pp. 46, 68). The ILV study author reported that, "Although the performing laboratory and sponsor are Syngenta, the ILV was conducted at different sites by different personnel." (p. 15). However, a statement specifying that the analysts and study director of the ILV were unfamiliar with the method, both in its development and use, should have been provided.

5. For the ILV, the individual peak area count data used to generate the standard curves were not reported and results from provided chromatograms of the standards were illegible (Figures 1-6, pp. 23-25; Figures 12-18, pp. 28-31; Figure 24, p. 34). The reviewer generated calibration curves using data (pg Analyte Found) from provided residue data sheets (Appendix 3, pp. 153-154; DER Attachment 2).

6. For the ECM, chromatograms were only provided for one calibration standard concentration (0.125 µg/L); the calibration standard curve range was 0.05-2.0 µg/L (Appendix 1, pp. 73-74, 79-80, 89-90, 105-106, 115-116).

7. The water matrices used in the ECM and ILV were characterized, but the primary sources of the matrices were not reported (p. 11; Table 1, p. 18; Appendix 1, p. 70).

8. In the ECM, triasulfuron and its product CGA150829 were found to be stable up to 9 days in final extracts stored at 0-9°C (Appendix 1, pp. 67, 75).

9. The ILV did not report the time required to complete a sample set (typically thirteen samples consisting of one reagent blank, two matrix control samples, and ten fortified samples). It

was reported in the ECM that analysis of a sample set consisting of fifteen samples required 1 working day (8 hours) per analyst (Appendix 1, pp. 58, 67).

V. References

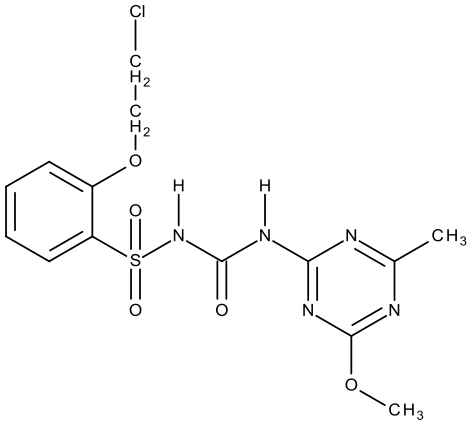
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

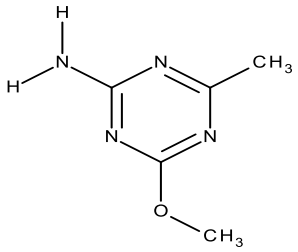
Triasulfuron (CGA131036)

IUPAC Name: 1-[2-(2-Chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea
CAS Name: 2-(2-Chloroethoxy)-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide
CAS Number: 82097-50-5
SMILES String: ClCCOc1ccccc1S(=O)(=O)NC(=O)Nc2nc(OC)nc(C)n2



CGA150829

IUPAC Name: 4-Methoxy-6-methyl-1,3,5-triazin-2-amine
CAS Name: Not reported
CAS Number: 1668-54-8
SMILES String: Cc1nc(nc(n1)OC)N



Attachment 2: Raw Data and Calculations

raw data and calcs