

Analytical method for fluthiacet-methyl and its metabolites CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in water

Reports: ECM: EPA MRID No.: Syngenta No. 1215-00. Chamkasem, N. 2001. Analytical Method for Determination of CGA-248757 and Its Degradates CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in Water by High Performance Liquid Chromatography with Mass Spectrometric Detection. Syngenta Laboratory Study ID No. 1215-00. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 114 pages. Final report issued January 9, 2001.

ILV: EPA MRID No. 50193201. Mannella, L. 2016. Independent Laboratory Validation of Analytical Methods for the Determination of Fluthiacet-methyl and Seven Metabolites in Water and Water. EAG Project No. 2812W. FMC Tracking No. 2015AMT-FLU2297. Report prepared by EAG Laboratories-Hercules (Formerly doing business as PTRL West), Hercules, California, sponsored and submitted by FMC Corporation, Ewing, New Jersey; 226 pages. Final report issued November 2, 2016.

Document No.: Syngenta No. 1215-00 & MRID 50193201

Guideline: 850.6100


Statements: ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception that the company Syngenta Crop Protection resulted from the spin-off of Novartis' AG Agribusiness and its subsequent merger with Zeneca Agrochemicals (40 CFR Part 160; p. 3 of Syngenta No. 1215-00). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). A certification of authenticity was not included.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3, 6 of MRID 50193201). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was included with the Quality Assurance statement.

Classification: This analytical method is classified as ACCEPTABLE. Significant matrix effects were observed, therefore matrix-matched standards must be used in application of this method. The study was not conducted to establish a true LOQ or LOD rather to confirm detection at specified value (i.e., reporting level).

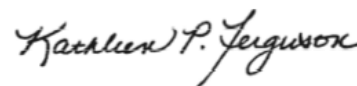
PC Code: 108803

EFED Final Reviewer: Gretchen E. Dykes, Biologist
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Date: 4/22/2021

CDM/CSS-Dynamac JV: Lisa Muto, M.S., Environmental Scientist
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Date: 3/22/18

Reviewers:Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature:



Date:

3/22/18

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Syngenta Report No. 1215-00, is designed for the quantitative determination of fluthiacet-methyl and its seven metabolites CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in water at the LOQ of 0.01 µg/L using LC/MS/MS. The study was not conducted to establish a true LOQ or LOD rather to confirm detection at specified value (i.e., reporting level). The LOQ was defined as the lowest concentration fortified in the control samples which yielded adequate recovery, and the LOD was defined as lowest amount of the standard injected. The LOQ (0.01 µg/L) is less than the lowest toxicological level of concern in water (1.4 µg a.i./L¹). The ECM validated the method using two uncharacterized water matrices; the ILV validated the method for all analytes using characterized pond water. The ILV validated for all analytes at both fortification levels in pond water after the first trial, with insignificant sample processing and analytical method modifications. Only one ion transition was monitored in the ECM while two ion transitions were monitored in the ILV for all analytes. A confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method. Based on the quantitation ion results, all ECM and ILV data was satisfactory regarding accuracy, precision, linearity and specificity, except for ECM performance data for CGA-300404 in one water matrix.

¹ Aquatic plants and algae EC₅₀ for vascular plants.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluthiacet- methyl (CGA- 248757)	Syngenta No. 1215-00	50193201	Acceptable 4/22/2021	Water ^{2,3}	09/01/2001	FMC Corporation	LC/MS/MS	0.01 µg/L
CGA- 300402								
CGA- 300403								
CGA- 300404								
CGA- 327066								
CGA- 327067								
CGA- 330057								
CGA- 330059								

1 Fluthiacet-methyl = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-methyl ester acetic acid; CGA-300402 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid; CGA-300403 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]thio]-acetic acid; CGA-300404 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; CGA-327066 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]thio]-acetic acid; CGA-327067 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]sulfinyl]-acetic acid; CGA-330057 = Methyl ester [[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H-3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; and CGA-330059 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]sulfinyl]-acetic acid.

2 In the ECM, the water matrices were obtained from Guilford County and Forsyth County, North Carolina, and used in the study (Appendix I, p. 103 of Syngenta No. 1215-00). The water characterization was not reported.

3 In the ILV, the pond water (Sample ID: 2706W-042; pH 6.3, 24 mg equivalent CaCO₃/L hardness, 4 ppm total dissolved solids) was obtained from Whaley Pond, Lenoir, Colorado, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, p. 212 of MRID 50193201).

I. Principle of the Method

Syngenta Report No. 1215-00

Water (300 mL) was measured into a 400-mL beaker, buffered with 1 mL of 43.5% ammonium acetate in water and 80 μ L of acetic acid, and fortified with a mixed standard solution of fluthiacet-methyl (CGA-248757) and its seven metabolites in acetonitrile:0.01 M ammonium acetate in water, pH 5 (10:90, v:v) for procedural recoveries (pp. 10-15, 19-20 of Syngenta No. 1215-00). The samples were applied to a Nexus Absolut Solid Phase Extraction (SPE) column (1 g, 6 mL; pre-conditioned with 6 mL of methanol followed by 10 mL of 0.05 M ammonium acetate in water, pH 5) via a plastic 75-mL reservoir on top of the SPE cartridge. The column should not be allowed to run dry. The sample beaker was rinsed with 2 x 5-10 mL of 0.05 M ammonium acetate, pH 5, which was applied to the column. The analytes were eluted from the column using *ca.* 10 mL acetonitrile into a 50-mL concentration tube calibrated to 3.0 mL. The calibration was done by adding 3.0 mL of 0.05 M ammonium acetate, pH 5, in water and marking the line with a waterproof fine-pointed pen. 0.5 mL of the 0.05 M ammonium acetate, pH 5, in water was added as a “water trap”. The method noted that CGA-248757 has been found to irreversibly bind to glassware when taken to dryness by rotary evaporation. The acetonitrile was removed from the sample until *ca.* 0.5 mL remains via rotary evaporator at a water bath temperature of *ca.* 25-30°C. 300 μ L of acetonitrile was added to the residue and vortex-mixed for *ca.* 1 min. The residue was diluted to 3 mL with 0.05 M ammonium acetate, pH 5, and vortex-mixed for *ca.* 30 seconds then sonicated for *ca.* 1 min. High-concentration samples were diluted, as necessary. Samples were analyzed by LC/MS/MS. The method noted that all laboratory equipment and glassware should be thoroughly cleaned prior to use. Additionally, several statements were made which indicated that the use of different water matrices, laboratory SPE columns and LC/MS/MS instruments could require optimization of the method parameters.

A Method Flow Diagram was included (Figure 2, p. 64 of Syngenta No. 1215-00).

Samples were analyzed using a Waters 2690 HPLC system and a Micromass Quattro Ultima MS with Ion-Spray interface (pp. 15-17; Tables 1-2, pp. 26-28 of Syngenta No. 1215-00). The following LC conditions were used: An Upchurch (A-318) pre-column filter (0.5 μ m), Phenomenex C-18 guard column, Develosil RPAQUEOUS-3 column (3.0 mm x 150 mm, 3 μ m, column temperature 30°C), mobile phase of (A) 0.1% acetic acid in acetonitrile and (B) 0.1% acetic acid in water [percent A:B (v:v) at 0 min. 10:90, 20 min. 70:30, 23-25 min. 100:0, 25.1-32 min. 10:90], and injection volume of 20 μ L. The following MS/MS conditions were used: positive electrospray ionization mode and multiple reaction monitoring (MRM). Analytes were identified using one ion pair transition as follows: m/z 404 \rightarrow 404 for fluthiacet-methyl (CGA-248757), m/z 390 \rightarrow 390 for CGA-300402, m/z 390 \rightarrow 344 for CGA-300403, m/z 406 \rightarrow 347 for CGA-300404, m/z 374 \rightarrow 356 for CGA-327066, m/z 390 \rightarrow 330 for CGA-327067, m/z 420 \rightarrow 347 for CGA-330057, and m/z 406 \rightarrow 346 for CGA-330059. Expected retention times are *ca.* 20.9, 17.5, 14.1, 12.7, 11.8, 7.9, 16.5, and 10.1 minutes for fluthiacet-methyl (CGA-248757), CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059, respectively.

ILV

The independent laboratory performed the ECM as written, except that a different Nexus column [Agilent Nexus SPE (0.1 g/12 mL)] was used, 2 mL of 0.05 M ammonium acetate in water (pH 5) was added to the Nexus column before the sample was loaded, matrix-based calibration standard solutions were used, and insignificant analytical method modifications (See Reviewer's Comment #1; pp. 21-22, 30-31, 33-34, 48 of MRID 50193201). An Agilent 1260 Series HPLC system and AB Sciex API 5500 Series Triple Quad MS was used. All LC and MS parameters were the same as the ECM, except that a confirmatory ion transition was also monitored for each analyte as follows: m/z 404→404 (Q) and m/z 404→344.1 (C) for fluthiacet-methyl (CGA-248757), m/z 390→390 (Q) and m/z 390→260 (C) for CGA-300402, m/z 390→344 (Q) and m/z 390→390 (C) for CGA-300403, m/z 406→347 (Q) and m/z 406→362 (C) for CGA-300404, m/z 374→356 (Q) and m/z 374→328 (C) for CGA-327066, m/z 390→330 (Q) and m/z 390→314 (C) for CGA-327067, m/z 420→347 (Q) and m/z 420→217 (C) for CGA-330057, and m/z 406→346 (Q) and m/z 406→344 (C) for CGA-330059 (the quantitation ion transitions were the same as the only monitored ion transitions of the ECM). Approximate retention times were 20.7, 17.5, 14.1, 13.1, 11.9, 8.2, 16.4, and 10.4 minutes for fluthiacet-methyl (CGA-248757), CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059, respectively. The ILV modifications did not warrant an updated ECM.

LOQ and LOD

In the ECM and ILV, Limit of Quantification (LOQ) for fluthiacet-methyl (CGA-248757), CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in water was 0.01 µg/L (pp. 10, 22, 25 of Syngenta No. 1215-00; pp. 42, 45 of MRID 50193201). The Limit of Detection (LOD) for all analytes in water was 10 pg (the lowest amount of standard injected; 0.0005 ng/µL calibration standard) in the ECM and 0.003 µg/L (30% of the LOQ) in the ILV.

II. Recovery Findings

ECM (Syngenta No. 1215-00): For Syngenta Method No. 1215-00, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of fluthiacet-methyl and its transformation products CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in two water matrices at fortification levels of 0.01 $\mu\text{g/L}$ (LOQ), 0.1 $\mu\text{g/L}$ (10 \times LOQ), and 1.0 $\mu\text{g/L}$ (100 \times LOQ), except for CGA-300404 in Guilford water at the LOQ, mean 125% (n = 4 for all analyses; Table 6, pp. 53-60; DER Attachment 2). One ion pair transition was monitored for each analyte; a confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method. Standard deviations and RSDs were reviewer-calculated based on data provided in the study report since the study author only provided overall statistics for the combined fortification data. The water matrices were obtained from Guilford County and Forsyth County, North Carolina, and used in the study (Appendix I, p. 103). The water characterization was not reported.

ILV (MRID 50193201): For Syngenta Method No. 1215-00, mean recoveries and RSDs were within guidelines for analysis for analysis of fluthiacet-methyl (CGA-248757) and its transformation products CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in one water matrix at fortification levels of 0.01 $\mu\text{g/L}$ (LOQ) and 0.1 $\mu\text{g/L}$ (10 \times LOQ; Table III, pp. 56-63). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable, except for the LOQ analysis of CGA-327067. The pond water (Sample ID: 2706W-042; pH 6.3, 24 mg equivalent CaCO_3/L hardness, 4 ppm total dissolved solids) was obtained from Whaley Pond, Lenoir, Colorado, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, p. 212). The method was validated for all analytes at both fortification levels in pond water after the first trial, with the following modifications: different Nexus column [Agilent Nexus SPE (0.1 g/12 mL)] was used, 2 mL of 0.05 M ammonium acetate in water (pH 5) was added to the Nexus column before the sample was loaded, matrix-based calibration standard solutions were used, and insignificant analytical method modifications (See Reviewer's Comment #1; pp. 21-22, 30-31, 33-34, 48).

Table 2. Initial Validation Method Recoveries for Fluthiacet-methyl (CGA-248757) and Its Transformation Products CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in Water^{1,2}

Analyte ³	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%) ⁴
Guilford Water						
Fluthiacet-methyl (CGA-248757)	0.01	4	93-101	96	3	4
	0.1	4	99-102	101	1	1
	1.0	4	98-105	102	3	3
CGA-300402	0.01	4	109-118	112	4	4
	0.1	4	104-108	106	2	2
	1.0	4	97-99	98	1	1
CGA-300403	0.01	4	104-114	110	5	4
	0.1	4	102-111	105	4	4
	1.0	4	89-95	91	3	3
CGA-300404	0.01	4	115-133	125	8	7
	0.1	4	101-104	103	1	1
	1.0	4	85-91	89	3	3
CGA-327066	0.01	4	111-126	117	7	6
	0.1	4	104-109	106	2	2
	1.0	4	92-95	93	2	2
CGA-327067	0.01	4	110-128	117	8	7
	0.1	4	96-107	102	5	5
	1.0	4	89-98	93	4	4
CGA-330057	0.01	4	101-112	105	5	5
	0.1	4	106-109	107	2	1
	1.0	4	94-96	95	1	1
CGA-330059	0.01	4	83-99	91	7	7
	0.1	4	93-99	96	3	3
	1.0	4	91-97	94	3	3
Forsyth Water						
Fluthiacet-methyl (CGA-248757)	0.01	4	78-93	83	7	8
	0.1	4	96-104	99	4	4
	1.0	4	96-104	100	4	4
CGA-300402	0.01	4	93-99	97	3	3
	0.1	4	94-105	98	5	5
	1.0	4	91-95	93	2	2
CGA-300403	0.01	4	86-99	95	6	6
	0.1	4	90-102	96	5	5
	1.0	4	87-89	88	1	1
CGA-300404	0.01	4	96-123	112	12	11
	0.1	4	99-114	108	7	7
	1.0	4	88-95	91	3	3
CGA-327066	0.01	4	91-115	100	11	11
	0.1	4	91-110	100	8	8
	1.0	4	93	93	0	0

Analyte ³	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%) ⁴
CGA-327067	0.01	4	94-103	100	4	4
	0.1	4	101-106	103	2	2
	1.0	4	89-96	93	3	3
CGA-330057	0.01	4	102-115	108	6	6
	0.1	4	101-108	105	3	3
	1.0	4	99-106	102	3	3
CGA-330059	0.01	4	67-90	77	10	13
	0.1	4	88-98	94	4	5
	1.0	4	88-96	92	3	4

Data (recovery results were corrected when residues were quantified in the controls; pp. 20-21) were obtained from Table 6, pp. 53-60 of Syngenta No. 1215-00 and DER Attachment 2.

- The water matrices were obtained from Guilford County and Forsyth County, North Carolina, and used in the study (Appendix I, p. 103). The water characterization was not reported.
- Analytes were identified using one ion pair transition as follows: m/z 404→404 for fluthiacet-methyl (CGA-248757), m/z 390→390 for CGA-300402, m/z 390→344 for CGA-300403, m/z 406→347 for CGA-300404, m/z 374→356 for CGA-327066, m/z 390→330 for CGA-327067, m/z 420→347 for CGA-330057, and m/z 406→346 for CGA-330059.
- Fluthiacet-methyl = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-methyl ester acetic acid; CGA-300402 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid; CGA-300403 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]thio]-acetic acid; CGA-300404 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; CGA-327066 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]thio]-acetic acid; CGA-327067 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]sulfinyl]-acetic acid; CGA-330057 = Methyl ester [[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H-3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; and CGA-330059 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]sulfinyl]-acetic acid.
- Standard deviations and relative standard deviations were reviewer-calculated based on data provided in the study report since the study author only provided overall statistics for the combined fortification data (see DER Attachment 2).

Table 3. Independent Validation Method Recoveries for Fluthiacet-methyl (CGA-248757) and Its Transformation Products CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in Water^{1,2}

Analyte ³	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Pond Water						
Quantitation Ion						
Fluthiacet-methyl (CGA-248757)	0.01 (LOQ)	5	82-92	88	4	5
	0.1	5	79-91	85	5	6
CGA-300402	0.01 (LOQ)	5	91-101	95	4	4
	0.1	5	89-94	91	2	2
CGA-300403	0.01 (LOQ)	5	81-93	87	5	6
	0.1	5	83-86	85	1	1
CGA-300404	0.01 (LOQ)	5	76-87	83	4	5
	0.1	5	85-91	88	3	3
CGA-327066	0.01 (LOQ)	5	76-100	82	11	13
	0.1	5	83-90	87	3	3
CGA-327067	0.01 (LOQ)	5	67-97	79	11	14
	0.1	5	85-92	88	3	3
CGA-330057	0.01 (LOQ)	5	86-92	89	3	3
	0.1	5	85-94	89	3	3
CGA-330059	0.01 (LOQ)	5	81-97	88	6	7
	0.1	5	80-84	82	2	2
Confirmation Ion						
Fluthiacet-methyl (CGA-248757)	0.01 (LOQ)	5	82-93	88	4	5
	0.1	5	80-91	85	5	6
CGA-300402	0.01 (LOQ)	5	90-95	93	2	2
	0.1	5	88-97	92	4	4
CGA-300403	0.01 (LOQ)	5	88-103	94	6	6
	0.1	5	79-86	82	3	4
CGA-300404	0.01 (LOQ)	5	76-93	87	7	8
	0.1	5	85-90	88	2	2
CGA-327066	0.01 (LOQ)	5	75-83	80	3	4
	0.1	5	83-86	85	2	2
CGA-327067	0.01 (LOQ)	5	89-123	102	14	14
	0.1	5	78-87	82	3	4
CGA-330057	0.01 (LOQ)	5	87-92	90	2	2
	0.1	5	85-93	88	3	3
CGA-330059	0.01 (LOQ)	5	67-90	76	9	12
	0.1	5	79-85	83	2	2

Data (recovery results were corrected when residues were quantified in the controls; p. 37) were obtained from Table III, pp. 56-63 of MRID 50193201.

1 The pond water (Sample ID: 2706W-042; pH 6.3, 24 mg equivalent CaCO₃/L hardness, 4 ppm total dissolved solids) was obtained from Whaley Pond, Lenoir, Colorado, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, p. 212).

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 404→404 (Q) and *m/z* 404→344.1 (C) for fluthiacet-methyl (CGA-248757), *m/z* 390→390 (Q) and *m/z* 390→260 (C) for CGA-300402, *m/z* 390→344 (Q) and *m/z* 390→390 (C) for CGA-300403, *m/z* 406→347 (Q) and *m/z* 406→362 (C) for CGA-300404, *m/z* 374→356 (Q) and *m/z* 374→328 (C) for CGA-327066, *m/z* 390→330 (Q) and *m/z* 390→314 (C) for

CGA-327067, m/z 420→347 (Q) and m/z 420→217 (C) for CGA-330057, and m/z 406→346 (Q) and m/z 406→344 (C) for CGA-330059 (the quantitation ion transitions were the same as the only monitored ion transitions of the ECM).

3 Fluthiacet-methyl = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-methyl ester acetic acid; CGA-300402 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid; CGA-300403 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]thio]-acetic acid; CGA-300404 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; CGA-327066 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]thio]-acetic acid; CGA-327067 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl]phenyl]sulfinyl]-acetic acid; CGA-330057 = Methyl ester [[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H-3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; and CGA-330059 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl]phenyl]sulfinyl]-acetic acid.

III. Method Characteristics

In the ECM and ILV, LOQ for fluthiacet-methyl (CGA-248757), CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in water were 0.01 µg/L (pp. 10, 22, 25 of Syngenta No. 1215-00; pp. 39-40, 42, 45 of MRID 50193201). In the ECM, the LOQ was defined as the lowest concentration fortified in the control samples which yielded adequate recovery. The LOD for all analytes in water was 10 pg (the lowest amount of standard injected; 0.0005 ng/µL calibration standard) in the ECM and 0.003 µg/L (30% of the LOQ; 0.3 ng/mL in the calibration standard solution using the current methodology) in the ILV. In the ECM and ILV, no calculations or comparisons of the LOQ or LOD to background noise were provided.

Table 4. Method Characteristics for Fluthiacet-methyl (CGA-248757) and Its Transformation Products CGA-300402, CGA-300403, CGA-300404, CGA-327066, CGA-327067, CGA-330057, and CGA-330059 in Water

Analyte ¹		CGA-248757	CGA-300402	CGA-300403	CGA-300404	CGA-327066	CGA-327067	CGA-330057	CGA-330059	
Limit of Quantitation (LOQ)	ECM	0.01 µg/L								
	ILV									
Limit of Detection (LOD) ²	ECM	10 pg (the lowest amount of standard injected; 0.0005 ng/µL calibration standard)								
	ILV	0.003 µg/L (30% of the LOQ; 0.3 ng/mL in the calibration standard solution using the current methodology)								
Linearity (calibration curve r ² and concentration range) ³	ECM ⁴	LOQ and 10×LOQ	r ² = 0.9998 (G) r ² = 0.9988 (F)	r ² = 0.9998 (G & F)	r ² = 0.9998 (G) r ² = 0.9990 (F)	r ² = 0.9990 (G) r ² = 0.9998 (F)	r ² = 0.9994 (G) r ² = 0.9998 (F)	r ² = 0.9992 (G) r ² = 0.9996 (F)	r ² = 0.9996 (G) r ² = 0.9998 (F)	r ² = 0.9998 (G & F)
		100×LOQ	r ² = 0.9988 (G & F)	r ² = 0.9988 (G & F)	r ² = 0.9994 (G & F)	r ² = 0.9996 (G & F)	r ² = 0.9972 (G & F)	r ² = 0.9966 (G & F)	r ² = 0.9998 (G & F)	r ² = 0.9998 (G & F)
	Range	0.01-0.20 ng (0.0005-0.01 ng/µL)								
	ILV		r ² = 0.9992 (Q) r ² = 0.9984 (C)	r ² = 0.9994 (Q & C)	r ² = 0.9980 (Q) r ² = 0.9916 (C)	r ² = 0.9962 (Q) r ² = 0.9946 (C)	r ² = 0.9950 (Q) r ² = 0.9984 (C)	r ² = 0.9990 (Q) r ² = 0.9972 (C)	r ² = 0.9996 (Q) r ² = 0.9992 (C)	r ² = 0.9986 (Q) r ² = 0.9958 (C)
		Range	0.3-10 ng/mL							
Repeatable	ECM ^{5,6}	Only quantitation ion transition monitored. ⁷								
		Yes at LOQ, 10×LOQ and 100×LOQ (two uncharacterized water matrices)	Yes at LOQ, 10×LOQ and 100×LOQ (F water). No at LOQ; yes at 10×LOQ and 100×LOQ (G water)	Yes at LOQ, 10×LOQ and 100×LOQ (two uncharacterized water matrices)						
	ILV ^{8,9}	Yes at LOQ and 10×LOQ (characterized pond water)								
Reproducible		Yes at LOQ and 10×LOQ								
Specific	ECM	Yes, matrix interferences were <5% (F) or ca. 14-19% (G) of LOQ (based on peak area).	Yes, no matrix interferences were observed.							

Analyte ¹		CGA-248757	CGA-300402	CGA-300403	CGA-300404	CGA-327066	CGA-327067	CGA-330057	CGA-330059
Specific	ILV	Yes, no matrix interferences were observed.			Yes, matrix interferences were <i>ca.</i> 5 of LOQ (based on peak area).	Yes, no matrix interferences were observed.		Yes, matrix interferences were <i>ca.</i> 1 of LOQ (based on peak area).	Yes, matrix interferences were 3-4% of LOQ (based on peak area).

Data were obtained from pp. 10, 22, 25; Table 6, pp. 53-60 (recovery results); Tables 3-5, pp. 29-52 (correlation coefficients); Figures 5-6, pp. 79-96 (chromatograms) of Syngenta No. 1215-00; pp. 39-40, 42, 45; Table III, pp. 56-63 (recovery results); Figure 5, pp. 104-119 (calibration curves); Figures 1, 3, 9-10, pp. 72-79, 88-95, 151-166 (chromatograms) of MRID 50193201; DER Attachment 2. G = Guilford Water; F = Forsyth Water. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Fluthiacet-methyl = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-methyl ester acetic acid; CGA-300402 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid; CGA-300403 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl)phenyl]thio]-acetic acid; CGA-300404 = [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; CGA-327066 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl)phenyl]thio]-acetic acid; CGA-327067 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl)phenyl]sulfinyl]-acetic acid; CGA-330057 = Methyl ester [[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H-3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid; and CGA-330059 = [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl)phenyl]sulfinyl]-acetic acid.

2 LOD reported in the ILV differed from that reported in the ECM.

3 ECM and ILV coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; Figure 8, pp. 58-63 of Syngenta No. 1215-00; Appendix 6, pp. 183-193 of MRID 49447003; DER Attachment 2). Solvent-based calibration standards.

4 The reviewer assumed that the linear regression was performed with 1/x weighting.

5 In the ECM, the water matrices were obtained from Guilford County and Forsyth County, North Carolina, and used in the study (Appendix I, p. 103 of Syngenta No. 1215-00). The water characterization was not reported.

6 Standard deviations and relative standard deviations were reviewer-calculated based on data provided in the study report since the study author did not report these values (see DER Attachment 2).

7 A confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method.

8 In the ILV, the pond water (Sample ID: 2706W-042; pH 6.3, 24 mg equivalent CaCO_3 /L hardness, 4 ppm total dissolved solids) was obtained from Whaley Pond, Lenoir, Colorado, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, p. 212 of MRID 50193201).

9 The ILV validated for all analytes at both fortification levels in pond water after the first trial, with the following modifications: different Nexus column [Agilent Nexus SPE (0.1 g/12 mL)] was used, 2 mL of 0.05 M ammonium acetate in water (pH 5) was added to the Nexus column before the sample was loaded, matrix-based calibration standard solutions were used, and insignificant analytical method modifications (See Reviewer's Comment #1; pp. 21-22, 30-31, 33-34, 48 of MRID 50193201).

Linearity is satisfactory when $r^2 \geq 0.995$.

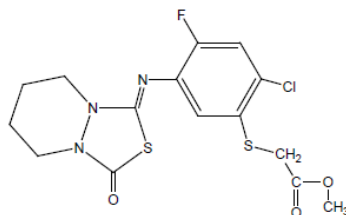
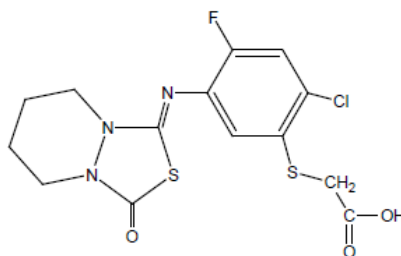
IV. Method Deficiencies and Reviewer's Comments

1. In the ILV modifications of the ECM, the ILV reported that the different Nexus column [Agilent Nexus SPE (0.5 g/12 mL)] was only used for the soil method, not the water method; however, the reviewer assumed that the different Nexus column was used for both methods based on the communications and since it was the only Nexus column listed in the equipment/materials list (pp. 21, 46, 48 of MRID 50193201).
2. ECM performance data was not satisfactory for CGA-300404 in Guilford water at the LOQ, mean 125% (n = 4; Table 6, pp. 53-60 of Syngenta No. 1215-00; DER Attachment 2). OCSPP guidelines state that mean recoveries and RSDs were within 70-120% and $\leq 20\%$, respectively. ECM performance data was satisfactory at 10 \times LOQ and 100 \times LOQ in the Guilford water and for the LOQ, 10 \times LOQ and 100 \times LOQ in the Forsyth water.
3. ILV linearity was not satisfactory for the confirmation ion calibration curves of CGA-300403 ($r^2 = 0.9916$) and CGA-300404 ($r^2 = 0.9946$; Figure 5, pp. 104-119 of MRID 50193201; DER Attachment 2). The reviewer noted that this deviation did not have an effect of the validity of the method since a confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method.
4. The ECM water matrices were not characterized, therefore it could not be determined if the ILV sandy loam water matrix was representative of TFD test waters.
5. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 22, 25 of Syngenta No. 1215-00; pp. 39-40, 42, 45 of MRID 50193201). In the ECM, the LOQ was defined as the lowest concentration fortified in the control samples which yielded adequate recovery. The LOD for all analytes in water was 10 pg (the lowest amount of standard injected; 0.0005 ng/ μ L calibration standard) in the ECM and 0.003 μ g/L (30% of the LOQ; 0.3 ng/mL in the calibration standard solution using the current methodology) in the ILV. In the ECM and ILV, no calculations or comparisons of the LOQ or LOD to background noise were provided. The LOD of the ECM differed from that of the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
6. The ILV study author provided communication details between the ILV laboratory personnel and the Sponsor Representative (Audrey Chen, FMC Corporation; pp. 3, 46-47 of MRID 50193201). These communications included communication of trial success or failure and approval of ILV modifications.
7. The reviewer noted that, although CGA-300403 was only identified and quantified using one ion pair transition versus in the ILV due to lack of a viable confirmation compound in the soil analysis, CGA-300403 was identified and quantified using two ion pair transitions in the ILV water analysis (p. 48; Table III, p. 58; Table IV, p. 66 of MRID 50193201).

8. The matrix effects were determined to be significant in the ILV (p. 43; Table I, pp. 51-52 of MRID 50193201). Matrix-matched standards were used. Solvent standards were used in the ECM (based on ILV modifications; p. 48).
9. It was reported for the ILV that a single analyst can complete a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) in *ca.* 19 hours (3 calendar days), with *ca.* 6 hours for preparation, *ca.* 9 hours for extraction and clean-up, and *ca.* 4 hours for LC/MS/MS analysis (pp. 40-41 of MRID 50193201).
10. In the ILV, the storage stability of the standard solutions was determined to be up to 108 days under refrigeration (4-10°C); the water extracts were determined to be stable for up to 16 days under refrigeration (4-10°C), except for CGA-327066 and CGA-327067 which were determined to be stable for up to 7 days under refrigeration (4-10°C; p. 45; Appendix E, pp. 224-225 of MRID 50193201).
11. In this ILV/ECM only three major degradates of fluthiacet-methyl were identified and quantified (CGA-300402, CGA-300403, and CGA-327066). No ECM/ILV exists for other major residues of concern, including IN VLH91-000, M7, M13, and VAP41-000.

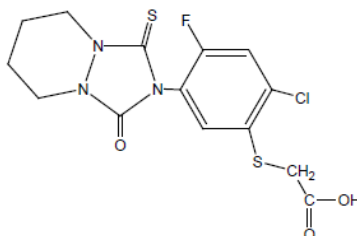
V. References

- 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.

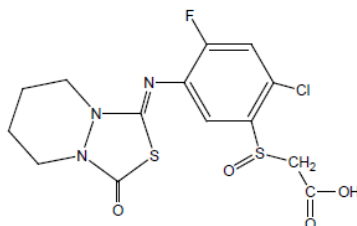
Attachment 1: Chemical Names and Structures**Fluthiacet-methyl (CGA-248757)****IUPAC Name:** Not reported**CAS Name:** [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-methyl ester acetic acid**CAS Number:** 117337-19-6**SMILES String:** Not found**CGA-300402****IUPAC Name:** Not reported**CAS Name:** [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid**CAS Number:** 149253-65-6**SMILES String:** Not found

CGA-300403

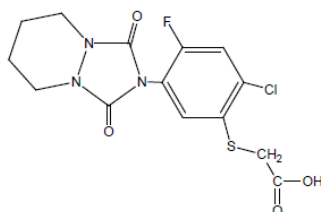
IUPAC Name: Not reported
CAS Name: [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl)phenyl]thio]-acetic acid
CAS Number: Not available
SMILES String: Not found

**CGA-300404**

IUPAC Name: Not reported
CAS Name: [[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid
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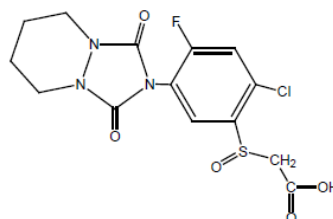
**CGA-327066**

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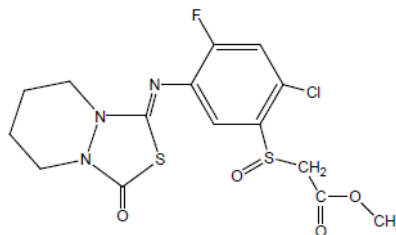


CGA-327067

IUPAC Name: Not reported
CAS Name: [[2-Chloro-4-fluoro-5-[(tetrahydro-1,3-dioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2-(3H)-yl)phenyl]sulfinyl]-acetic acid
CAS Number: Not available
SMILES String: Not found

**CGA-330057**

IUPAC Name: Not reported
CAS Name: Methyl ester [[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H-3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfinyl]-acetic acid
CAS Number: Not available
SMILES String: Not found



CGA-330059**IUPAC Name:** Not reported**CAS Name:** [[2-Chloro-4-fluoro-5-[(tetrahydro-1-oxo-3-thioxo-1H-[1,2,4]triazolo[1,2-a]pyridazin-2(3H)-yl)phenyl]sulfinyl]-acetic acid**CAS Number:** Not available**SMILES String:** Not found