Analytical method for flumetralin in soil

Reports: ECM: EPA MRID No.: 50213302. Mayer, L.C. 2017. Flumetralin. Flumetralin - Analytical Method GRM060.08A for the Determination of Flumetralin (CGA41065) in Soil by GC-NICI-MSD (Version 2 of MRID 50213302). – Analytical Method. Syngenta Report No. GRM060.08A and Task No. TK0310460. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 37 pages. Final report issued October 27, 2017.

ILV: EPA MRID No. 50449302. Xu, A. 2017. Flumetralin. Independent Laboratory Validation of "Flumetralin – Analytical Method GRM060.08A for the Determination of Flumetralin (CGA41065) in Soil by GC-NICI-MSD." Final ILV Report. Report No.: PASC-REP-1385. PASC Project No.: 141-2220. Task No.: TK0309730. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 123 pages. Final report issued November 20, 2017.

Document No.: MRIDs 50213302 & 50449302

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (p. 3 of MRID 50213302). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Previous Versions was included (p. 4).
ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50449302). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was not included.

Classification: This analytical method is classified as unacceptable. It could not be determined if the ILV was conducted independently of the ECM since the ILV study author communicated directly with Louis Mayer of Syngenta who was the ECM study author, as well as the ILV Study Monitor. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method and that ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. More details about the extraction procedure and analytical instruments should have been reported in the ILV. In the ECM, the purity of the test material was not reported.

PC Code: 123001

EFED Final Reviewer:	Ibrahim Abdel-Saheb, Ph.D., Environmental Scientist	Signature: Date: 2/8/2019
CDM/CSS-Dynamac JV	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 10/18/2018

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Signature: Mary Samuel

Date: 10/25/2018

Executive Summary

This analytical method, Syngenta Residue Method GRM060.08A, is designed for the quantitative determination of flumetralin (CGA41065) in soil at the LOQ of 0.01 mg/kg using GC/MS. The ECM and ILV used one different characterized soil matrix each. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method and that ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. Three ions were monitored, but results were only provided for the primary ion. A confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. **It could not be determined if the ILV was conducted independently of the ECM** since the ILV study author communicated directly with the ECM study author. The reviewer assumed that the ILV validated the ECM in the first trial with no or insignificant modifications. Only a brief summary of the method was included in the ILV; more details about the extraction procedure and analytical instruments/parameters should have been reported in the ILV to compare methods. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory. In the ECM, the purity of the test material was not reported.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Flumetralin (CGA41065)	50213302 (GRM060.08A)	50449302		Soil[1,2]	27/10/2017	Syngenta Crop Protection, LLC	GC/MS	0.01 mg/kg

1 In the ECM, the clay loam soil (18/46/36 sand/silt/clay, pH 5.7 in 0.01M $CaCl_2$, 1.8% organic carbon) was used in the study (USDA soil texture characterization not specified; Table 1, p. 21 of MRID 50213302). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

2 In the ILV, the loamy sand soil (Sample ID 170884-2 500 G; 75/20/5 sand/silt/clay, pH 5.9 in 1:1 soil:water ratio, 7.4% organic matter – Walkley Black) was used in the study (USDA soil texture characterization; p. 10; Table 1, p. 15 of MRID 50449302). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

I. Principle of the Method

Syngenta Residue Method GRM060.08A

Soil (10 g) in 150-mL polypropylene bottles was fortified with flumetralin in acetone for procedural recoveries (pp. 9-12; Appendix 4, p. 37 of MRID 50213302). The samples were mixed with 100 mL of methanol:water (80:20, v:v) via mechanical shaker [275 rpm (or at a speed that visibly agitates the samples) for 2 hours]. After centrifugation (3500 rpm for 5 minutes), an aliquot (5 mL) of the organic layer was transferred to a 50-mL polypropylene centrifuge tube. The sample was mixed with 15 mL of aqueous saturated sodium chloride and 5 mL of hexane:toluene (50:50, v:v) via mechanical shaker (275 rpm for 10 minutes). After centrifugation (3500 rpm for 5 minutes), an aliquot (1 mL) of the organic layer was transferred to a 15-mL polypropylene centrifuge tube and diluted to 4 mL with hexane:toluene (50:50, v:v). An aliquot was transferred to an autosampler vial for GC/MS analysis. Further dilutions with hexane:toluene (50:50, v:v) can be performed based on instrument sensitivity.

Samples are analyzed using an Agilent 7890B GC coupled to a 5977B MSD (pp. 13-14; Appendix 1, p. 34; Appendix 3, p. 36 of MRID 50213302). The following conditions were used: HP-5MS column (30.0 m x 0.25 mm, 0.25 µm), helium carrier gas, injector temperature 250°C, ion source and quadrupole temperature 150°C, temperature program 120°C for 1 minute to 300°C for 2 minutes (rate 20°C/min.), chemical SIM ionization mode in negative polarity. Injection volume was 2 µL. Expected retention time for flumetralin is *ca*. 9.3 minutes. Flumetralin was identified using three ions (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 421, 423, and 391.

ILV

The ILV reportedly performed Syngenta Residue Method GRM060.08A with GC/MS using negative-ion chemical ionization as written; however, only a brief summary of the method was included, which did not include the addition of 15 mL of aqueous saturated sodium chloride and the specific analytical instruments and parameters (pp. 9-11, 13 of MRID 50449302). However, the ILV reported that the validation was performed using the procedures and instruments recommended by the method. Flumetralin was identified using the same three ions as those reported in the ECM; expected retention time was *ca*. 8.46 minutes (Figures 2-7, pp. 20-25).

In the ECM and ILV, the Limit of Quantification (LOQ) for flumetralin in Syngenta Residue Method GRM060.08A was reported as 0.01 mg/kg (ppm; pp. 9, 17-18 of MRID 50213302; pp. 8, 11 of MRID 50449302). The Limit of Detection (LOD) for flumetralin was 0.5 pg injected on column, equivalent to 0.25 pg/µL, when using a 2 µL injection in the ECM and the ILV.

II. Recovery Findings

ECM (MRID 50213302): For Syngenta Residue Method GRM060.08A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of flumetralin at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg) in one soil matrix (Table 2, p. 21; DER Attachment 2). Three ions were monitored via GC/MS analysis; performance data (results) was only provided for the primary ion. A confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. The clay loam soil (18/46/36 sand/silt/clay, pH 5.7 in 0.01M $CaCl_2$, 1.8% organic carbon) was used in the study (USDA soil texture characterization not specified; Table 1, p. 21). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

ILV (MRID 50449302): For Syngenta Residue Method GRM060.08A, mean recoveries and RSDs were within guidelines for analysis of flumetralin at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg) in one soil matrix (p. 12; Table 3, p. 17). Three ions were monitored via GC/MS analysis; performance data (results) was only provided for the primary ion. The loamy sand soil (Sample ID 170884-2 500 G; 75/20/5 sand/silt/clay, pH 5.9 in 1:1 soil:water ratio, 7.4% organic matter – Walkley Black) was used in the study (USDA soil texture characterization; p. 10; Table 1, p. 15). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported. The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.08A with GC/MS using negative-ion chemical ionization in the first trial with no or insignificant modifications (p. 8). Only a brief summary of the method was included in the ILV, which did not include the addition of 15 mL of aqueous saturated sodium chloride and the specific analytical instruments and parameters (pp. 9-11, 13). However, the ILV reported that the validation was performed using the procedures and instruments recommended by the method.

Table 2. Initial Validation Method Recoveries for Flumetralin in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Clay Loam Soil					
	Primary ion					
Flumetralin	0.01	5	106-119	113	5	4.4
	0.1	5	114-126	118	5	3.9

Data (uncorrected recovery results; pp. 14-15) were obtained from Table 2, p. 21 of MRID 50213302 and DER Attachment 2.

1 The clay loam soil (18/46/36 sand/silt/clay, pH 5.7 in 0.01M $CaCl_2$, 1.8% organic carbon) was used in the study (USDA soil texture characterization not specified; Table 1, p. 21). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

2 Flumetralin was identified using three ions (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 421, 423, and 391; however, recovery results were only reported for the primary ion.

3 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). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Flumetralin in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil					
	Primary ion					
Flumetralin	0.01	5	84-93	87	3.4	3.9
	0.1	5	94-101	100	4.3	4.3

Data (uncorrected recovery results; Appendix 3, p. 78) were obtained from p. 12; Table 3, p. 17 of MRID 50449302.

1 The loamy sand soil (Sample ID 170884-2 500 G; 75/20/5 sand/silt/clay, pH 5.9 in 1:1 soil:water ratio, 7.4% organic matter – Walkley Black) was used in the study (USDA soil texture characterization; p. 10; Table 1, p. 15). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

2 Flumetralin was identified using three ions (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 421, 423, and 391; however, recovery results were only reported for the primary ion.

III. Method Characteristics

In the ECM and ILV, the LOQ for flumetralin in Syngenta Residue Method GRM060.08A was reported as 0.01 mg/kg (ppm; pp. 9, 17-18 of MRID 50213302; pp. 8, 11 of MRID 50449302). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. The LOD for flumetralin was 0.5 pg injected on column, equivalent to 0.25 pg/µL, when using a 2 µL injection in the ECM and the ILV. In the ECM and ILV, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise The ECM and ILV study authors noted that the LOD may vary between runs and from instrument to instrument. No calculations for LOQ and LOD were reported in the ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Table 4. Method Characteristics for Flumetralin in Soil[1]

Analyte		**Flumetralin**
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg
	ILV	
Limit of Detection (LOD)	ECM	0.5 pg injected on column, equivalent to 0.25 pg/µL, when using a 2 µL injection
	ILV	
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.99938791$
	ILV	$r^2 = 0.997308$
	Range	0.25-10.0 ng/mL
Repeatable	ECM[2]	Yes at LOQ and 10×LOQ.
	ILV[3,4]	Yes at LOQ and 10×LOQ.
Reproducible		Yes at LOQ and 10×LOQ.
Specific	ECM	Yes, matrix interferences were <1% of the LOQ (based on quantified residues).
	ILV	Yes, no matrix interferences were observed at the analyte RT.

Data were obtained from pp. 9, 17-18 (LOQ/LOD); Table 2, p. 21 (recovery results); Table 5, p. 24 (calibration data); Figure 8, p. 30 (calibration curve); Figures 9-12, pp. 31-32 (chromatograms) of MRID 50213302; pp. 8, 11 (LOQ/LOD); p. 12; Table 3, p. 17 (recovery results); Figures 8-11, pp. 26-29 (chromatograms); Figure 12, p. 30 (calibration curves) of MRID 50449302. All results refer to the primary ion only.

1 Three ions were monitored via GC/MS analysis; performance data (results) was only provided for the primary ion. A confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

2 In the ECM, the clay loam soil (18/46/36 sand/silt/clay, pH 5.7 in 0.01 M $CaCl_2$, 1.8% organic carbon) was used in the study (USDA soil texture characterization not specified; Table 1, p. 21 of MRID 50213302). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

3 In the ILV, the loamy sand soil (Sample ID 170884-2 500 G; 75/20/5 sand/silt/clay, pH 5.9 in 1:1 soil:water ratio, 7.4% organic matter – Walkley Black) was used in the study (USDA soil texture characterization; p. 10; Table 1, p. 15 of MRID 50449302). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil source was not reported.

4 The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.08A with GC/MS using negative-ion chemical ionization in the first trial with no or insignificant modifications (p. 8 of MRID 50449302). Only a brief summary of the method was included in the ILV, which did not include the addition of 15 mL of aqueous saturated sodium chloride and the specific analytical instruments and parameters (pp. 9-11, 13). However, the ILV reported that the validation was performed using the procedures and instruments recommended by the method.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was conducted independently of the ECM since the ILV study author communicated directly with Louis Mayer of Syngenta who was the ECM study author, as well as the ILV Study Monitor (pp. 5, 13; Appendix 5, pp. 116-123 of MRID 50449302). These communications included exchange of protocols, and notifications of successful trials; however, the ILV study author also reported the suspected problem with the first trial and requested the ECM study author's approval for the solution to the problem before beginning the second trial. The ECM study author requested details about the problem which occurred in the water validation and provided approval for the ILV solution. OCSPP guidelines state that ILV validations are performed without collusion with the ECM personnel. The reviewer noted that the ILV study report stated that no communication about the method was conducted during the ILV validation (p. 13).

   The reviewer also noted that the ILV provided their own matrices for the validations (Appendix 5, pp. 116-123 of MRID 50449302).

2. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method since only one soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Additionally, since no terrestrial field dissipation studies were submitted, it not be determined if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies.

3. The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.08A with GC/MS using negative-ion chemical ionization in the first trial with no or insignificant modifications (p. 8 of MRID 50449302). Only a brief summary of the method was included in the ILV, which did not include the addition of 15 mL of aqueous saturated sodium chloride and the specific analytical instruments and parameters (pp. 9-11, 13). However, the ILV reported that the validation was performed using the procedures and instruments recommended by the method. The reviewer believed that more details about the extraction procedure and analytical instruments/parameters should have been reported in the ILV to compare methods.

4. The purity of the test material was not reported in the ECM (Figure 1, p. 26; Appendix 2, p. 35 of MRID 50213302).

5. USDA soil texture characterization not specified for the ECM soil matrix, although soil characterization was performed by Agvise Laboratories, Northwood, North Dakota (Table 1, p. 21 of MRID 50213302).

6. 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. 9, 17-18 of MRID 50213302; pp. 8, 11 of MRID 50449302). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. In the ECM and ILV, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise The ECM and ILV study authors noted that the LOD may vary between runs and from instrument to instrument. No calculations for LOQ and LOD were reported in the ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   The ECM reported the most sensitive toxicity endpoint for terrestrial plants NOAEC = 0.019 lb a.i./A (19 ppb based on 3-inch core; p. 17 of MRID 50213302).

7. In the ECM, the matrix effects were determined to be insignificant (<±20%; p. 18; Table 3, p. 22 of MRID 50213302). Solvent standards were used.

8. In the ECM, the final soil extracts were found to be stable for up to *ca*. 7 days at *ca*. 4°C (Table 4, p. 23 of MRID 50213302).

9. The ECM reported that 1 sample set of 12 samples each can be completed in 1 day (8 hour working period) by one analyst (p. 12 of MRID 50213302). The time required to complete the method was not reported in the ILV.

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

Flumetralin (CGA41065)

IUPAC Name:	N-(2-chloro-6-fluorobenzyl)-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine
CAS Name:	2-Chloro-N-[2,6-dinitro-4-(trifluoromethyl)phenyl]-N-ethyl-6-fluorobenzenemethanamine
CAS Number:	62924-70-3
SMILES String:	N(=O)(=O)c1cc(C(F)(F)F)cc(N(=O)(=O))c1N(CC)Cc2c(F)cccc2Cl