

Analytical method for flumetralin in water

Reports: ECM: EPA MRID No.: 50213301. Mayer, L.C. 2017. Flumetralin. Flumetralin - Analytical Method GRM060.09A for the Determination of Flumetralin (CGA41065) in Water by GC-NICI-MSD (Version 2 of MRID 50213301). Analytical Method. Syngenta Report No. GRM060.09A and Task No. TK0310459. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 47 pages. Final report issued October 27, 2017.

ILV: EPA MRID No. 50449301. Xu, A. 2017. Flumetralin. Independent Laboratory Validation of “Flumetralin – Analytical Method GRM060.09A for the Determination of Flumetralin (CGA41065) in Water by GC-NICI-MSD.” Final ILV Report. Report No.: PASC-REP-1384. PASC Project No.: 141-2219. Task No.: TK0309726. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 147 pages. Final report issued November 20, 2017.


Document No.: MRIDs 50213301 & 50449301


Guideline: 850.6100


Statements: ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (p. 3 of MRID 50213301). 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 50449301). 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. ILV linearity was not satisfactory for flumetralin. 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.,
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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

This analytical method, Syngenta Residue Method GRM060.09A, is designed for the quantitative determination of flumetralin (CGA41065) in water at the LOQ of 0.05 µg/L using GC/MS. The ECM and ILV used two different characterized water matrices each. 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 about the problem in the first trial. The reviewer assumed that the ILV validated the ECM in the second trial with no or insignificant modifications. The first trial reportedly failed due to the very small fortification volume used; a larger volume was used for the successful trial. This was not a modification of the ECM. 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, except for ILV linearity. 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)	50213301 (GRM060.09A)	50449301		Water ^{1,2}	27/10/2017	Syngenta Crop Protection, LLC	GC/MS	0.05 µg/L

1 In the ECM, the ground water (pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃), obtained from Summerfield, and surface water (pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃), obtained from Belews Lake, were used in the study (Table 1, p. 24 of MRID 50213301). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not further described.

2 In the ILV, the ground water (Sample ID 170882 1000 ML; pH 7.7, 122 ppm total dissolved solids, 125 mg/L total hardness as CaCO₃) and surface water (Sample ID 170883 1000 ML; pH 8.3, 116 ppm total dissolved solids, 112 mg/L total hardness as CaCO₃) were used in the study (p. 10; Tables 1-1 and 1-2, pp. 16-17 of MRID 50449301). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not reported.

I. Principle of the Method

Syngenta Residue Method GRM060.09A

Water (15 mL) in 50-mL polypropylene centrifuge tubes was fortified with flumetralin in acetone for procedural recoveries (pp. 11, 13; Appendix 4, p. 47 of MRID 50213301). The samples were 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 an autosampler vial for GC/MS analysis. Further dilutions with hexane:toluene (50:50, v:v) can be performed based on instrument sensitivity.

The method contained the following precautions to minimize carry-over issues: high-level recovery samples should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL and solvent blank injections should be included after high-level samples to clear observed carry-over >10% of the LOQ (p. 13 of MRID 50213301).

Samples are analyzed using an Agilent 7890B GC coupled to a 5977B MSD (pp. 14-15; Appendix 1, p. 44; Appendix 3, p. 46 of MRID 50213301). 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 GRM060.09A 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. 10-11, 13 of MRID 50449301). 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 2-4, pp. 22-24).

In the ECM and ILV, the Limit of Quantification (LOQ) for flumetralin in Syngenta Residue Method GRM060.09A was reported as 0.05 µg/L (ppm; pp. 10, 18-19, 21 of MRID 50213301; pp. 8, 11 of MRID 50449301). The Limit of Detection (LOD) for flumetralin was 0.1 pg injected on column, equivalent to 0.05 µg/L (0.05 pg/µL), when using a 2 µL injection in the ECM and the ILV.

II. Recovery Findings

ECM (MRID 50213301): For Syngenta Residue Method GRM060.09A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of flumetralin at the LOQ (0.05 $\mu\text{g/L}$) and 10 \times LOQ (0.5 $\mu\text{g/L}$) in two water matrices (Table 2, p. 24; 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 ground water (pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO_3), obtained from Summerfield, and surface water (pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO_3), obtained from Belews Lake, were used in the study (Table 1, p. 24). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not further described.

ILV (MRID 50449301): For Syngenta Residue Method GRM060.09A, mean recoveries and RSDs were within guidelines for analysis of flumetralin at the LOQ (0.05 $\mu\text{g/L}$) and 10 \times LOQ (0.5 $\mu\text{g/L}$) in two water matrices (p. 12; Table 3, p. 19). Three ions were monitored via GC/MS analysis; performance data (results) was only provided for the primary ion. The ground water (Sample ID 170882 1000 ML; pH 7.7, 122 ppm total dissolved solids, 125 mg/L total hardness as CaCO_3) and surface water (Sample ID 170883 1000 ML; pH 8.3, 116 ppm total dissolved solids, 112 mg/L total hardness as CaCO_3) were used in the study (p. 10; Tables 1-1 and 1-2, pp. 16-17). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not reported. The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.09A with GC/MS using negative-ion chemical ionization in the second trial with no or insignificant modifications (p. 8). The first trial reportedly failed due to the very small fortification volume used (75 μL); a larger volume (0.5 mL) was used for the successful trial (Appendix 5, pp. 140-141). This was not a modification of the ECM since the ECM did not specify the fortification volume and only stated that it should be <1 mL (p. 13 of MRID 50213301). 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. 10-11, 13 of MRID 50449301). 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 Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Ground Water						
Primary ion						
Flumetralin	0.05	5	87-95	91	3	3.5
	0.5	5	94-108	101	5	5.0
Surface Water						
Primary ion						
Flumetralin	0.05	5	87-102	95	6	6.4
	0.5	5	98-108	104	4	3.9

Data (recovery results were corrected for residues quantified in the controls; p. 16) were obtained from Table 2, p. 24 of MRID 50213301 and DER Attachment 2.

- 1 The ground water (pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃), obtained from Summerfield, and surface water (pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃), obtained from Belews Lake, were used in the study (Table 1, p. 24). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not further described.
- 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 Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Primary ion						
Flumetralin	0.05	5	90-110	104	8.1	7.8
	0.5	5	99-106	103	3.0	2.9
Surface Water						
Primary ion						
Flumetralin	0.05	5	87-129	110	14.9	14
	0.5	5	102-117	109	6.4	5.9

Data (uncorrected recovery results; Appendix 3, p. 92) were obtained from p. 12; Table 3, p. 19 of MRID 50449301.

- 1 The ground water (Sample ID 170882 1000 ML; pH 7.7, 122 ppm total dissolved solids, 125 mg/L total hardness as CaCO₃) and surface water (Sample ID 170883 1000 ML; pH 8.3, 116 ppm total dissolved solids, 112 mg/L total hardness as CaCO₃) were used in the study (p. 10; Tables 1-1 and 1-2, pp. 16-17). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were 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 GRM060.09A was reported as 0.05 µg/L (ppm; pp. 10, 18-19, 21 of MRID 50213301; pp. 8, 11 of MRID 50449301). 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 $\leq 20\%$. The LOD for flumetralin was 0.1 pg injected on column, equivalent to 0.05 µg/L (0.05 pg/µL), when using a 2 µL injection in the ECM and 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 Water¹

Analyte		Flumetralin
Limit of Quantitation (LOQ)	ECM	0.05 µg/L
	ILV	
Limit of Detection (LOD)	ECM	0.1 pg injected on column, equivalent to 0.05 µg/L (0.05 pg/µL), when using a 2 µL injection
	ILV	
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.99938208$
	ILV	$r^2 = \mathbf{0.994962}$
	Range	0.05-10.0 pg/µL
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 in the surface water and <10% of the LOQ in the ground water (based on quantified residues).
	ILV	Yes, no matrix interferences were observed at the analyte RT, but some contaminants were observed with peak ht. \geq LOQ peak height.

Data were obtained from pp. 10, 18-19, 21 (LOQ/LOD); Table 2, p. 24 (recovery results); Table 5, p. 27 (calibration data); Figure 8, p. 36 (calibration curve); Figures 9-14, pp. 37-42 (chromatograms) of MRID 50213301; pp. 8, 11 (LOQ/LOD); p. 12; Table 3, p. 19 (recovery results); Figures 8-14, pp. 28-34 (chromatograms); Figure 15, p. 35 (calibration curves) of MRID 50449301. 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 ground water (pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃), obtained from Summerfield, and surface water (pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃), obtained from Belews Lake, were used in the study (Table 1, p. 24 of MRID 50213301). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not further described.

3 In the ILV, the ground water (Sample ID 170882 1000 ML; pH 7.7, 122 ppm total dissolved solids, 125 mg/L total hardness as CaCO₃) and surface water (Sample ID 170883 1000 ML; pH 8.3, 116 ppm total dissolved solids, 112 mg/L total hardness as CaCO₃) were used in the study (p. 10; Tables 1-1 and 1-2, pp. 16-17 of MRID 50449301). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water sources were not reported.

4 The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.09A with GC/MS using negative-ion chemical ionization in the second trial with no or insignificant modifications (p. 8 of MRID 50449301). The first trial reportedly failed due to the very small fortification volume used (75 µL); a larger volume (0.5 mL) was used for the successful trial (Appendix 5, pp. 140-141). This was not a modification of the ECM since the ECM did not specify the fortification volume and only stated that it should be <1 mL (p. 13 of MRID 50213301). 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. 10-11, 13 of MRID 50449301). However, the ILV reported that the validation was performed using the procedures and instruments recommended by the method.

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

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. 140-147 of MRID 50449301). 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. 140-147 of MRID 50449301).

2. ILV linearity was not satisfactory for flumetralin, $r^2 = 0.994962$ (Figure 15, p. 35 of MRID 50449301). Linearity is satisfactory when $r^2 \geq 0.995$.
3. The reviewer assumed that the ILV validated Syngenta Residue Method GRM060.09A with GC/MS using negative-ion chemical ionization in the second trial with no or insignificant modifications (p. 8 of MRID 50449301). The first trial reportedly failed due to the very small fortification volume used (75 μ L); a larger volume (0.5 mL) was used for the successful trial (Appendix 5, pp. 140-141). This was not a modification of the ECM since the ECM did not specify the fortification volume and only stated that it should be <1 mL (p. 13 of MRID 50213301). 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. 10-11, 13 of MRID 50449301). 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. 29; Appendix 2, p. 45 of MRID 50213301).
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, 18-19, 21 of MRID 50213301; pp. 8, 11 of MRID 50449301). 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 $\leq 20\%$. 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 aquatic organisms NOAEC = 0.77 µg/L (Flathead minnow; p. 19 of MRID 50213301).

6. In the ECM, the matrix effects were determined to be insignificant ($<\pm 20\%$; p. 19; Table 3, p. 25 of MRID 50213301). Solvent standards were used.
7. In the ECM, the final water extracts were found to be stable for up to *ca.* 7 days at *ca.* 4°C (p. 19; Table 4, p. 26 of MRID 50213301).
8. The ECM reported that 1-2 sample sets of 20 samples each can be completed in 1 day (8 hour working period) by one skilled analyst (p. 13 of MRID 50213301). 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

