Analytical method for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in Water

Reports: ECM 1: EPA MRID No.: 51458738. Wang M. 2019. An Analytical Method for the Determination of Residues of AE F088657 and its metabolites AE 0542291 and AE B107137 in Water Using LC/MS/MS. Analytical Method. Report prepared by Bayer CropScience, Research Triangle Park, North Carolina, and sponsored and submitted by Bayer CropScience LP, St. Louis, Missouri; 25 pages. Bayer Method No.: DC-004-W19-01. Activity ID: RADC0097. Final report issued February 20, 2019.

ECM 2: EPA MRID No.: 51458739. Wang M. 2019. In House Laboratory Validation of an Analytical Method for the Determination of Residues of AE F088657 and its Metabolites AE 0542291 and AE B107137 in Water Using LC/MS/MS. Final Report. Report prepared by Bayer CropScience LP, Chesterfield, Missouri, and sponsored and submitted by Bayer CropScience LP, St. Louis, Missouri; 88 pages. Activity/Study ID: RADC0097. Final report issued October 14, 2019.

ILV: EPA MRID No.: 51458740. Miner, P. 2019. "Independent Laboratory Validation of Analytical Method DC-004-W19-01 for the Determination of Residues of AE F088657 and its Metabolites AE 0542291 and AE B107137 in Water". Final Report. Report prepared by Frontage Laboratories, Concord, Ohio, and sponsored and submitted by Bayer CropScience, St. Louis, Missouri; 88 pages. Laboratory Study ID: 037617. Sponsor Study ID: RADC0094. Final report issued October 31, 2019.

Document No.: MRIDs 51458738 & 51458739 & 51458740

Guideline: 850.6100

Statements: ECM 1: The report was not conducted in compliance with USEPA FIFRA Good Laboratory (GLP) standards (40 CFR Part 160) or OECD GLP since it was not a study (p. 3 of MRID 51458738). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included.
ECM 2: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; 1989; p. 3 of MRID 51458739). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). An Authenticity statement was not included.
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 51458740). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as supplemental. ECM 1 MRID 51458738 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51458739 contained performance data, but only a method summary. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The specificity of the method for AE B107137 was not supported by ILV

	representative chromatograms. The ILV used one uncharacterized water matrix, while the ECM used two uncharacterized water matrices. The number of ILV trials required to validate the method was not reported. The ECM method (Bayer Method No. DC-004-W19-01) may need to be updated with precautions against the use of tap or 'purified' water.		
PC Code:	128009		
EFED Final Reviewer:	Sheng Lin, Ph.D., Physical Scientist	Signature: Date:	
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	Lisa Muto 10/08/2021
	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	Marysamuel 10/08/2021

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, Bayer Method No. DC-004-W19-01, is designed for the quantitative determination of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in water at the stated LOQ of 0.05 ng/mL using LC-MS/MS. The LOQ is less than the lowest toxicological level of concern in water for diflufenican, AE 0542291, and AE B107137. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM 2, the LLMV was equivalent to the ECM reported method LOQ for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in the tested water matrices (0.05 ng/mL). ECM 1 MRID 51458738 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51458739 contained performance data, but only a method summary.

The ECM validated the method using two uncharacterized water matrices (ground water and "grocery store water"); the ILV validated the method using one uncharacterized water matrix (pond water). The number of ILV trials required to validate the method (Bayer Method No. DC-004-W19-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for water was not reported; however, it was assumed that the ILV validated the method in the first trial as written with insignificant modifications to the analytical parameters. The ILV modifications did not warrant an updated ECM; however, Bayer Method No. DC-004-W19-01 may need to be updated with precautions against the use of tap or 'purified' water based on communications between the ILV Study Author and Study Monitor.

All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for diflufenican, AE 0542291, and AE B107137 in the test water matrix, except that the specificity of the method for AE B107137 was not supported by ILV representative chromatograms due to significant baseline noise around the LOQ analyte peak and the fact that the LOQ analyte peak response was only slightly greater than matrix contaminants. All ECM 2 data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for diflufenican, AE 0542291, and AE B107137 in both test water matrices.

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						
Diflufenican (AE F088657)	51458738[1] & 51458739[2]	51458740[3]		Water	20/02/2019 (Method) 14/10/2019 (Internal validation)	Bayer CropScience	LC-MS/MS	0.05 ng/mL
AE 0542291 (Phenoxynicotinamide)								
AE B107137 (Diflufenican acid)								

1 ECM 1 MRID 51458738 was a method only study report. No performance data was submitted in this study.
2 In the ECM 2 MRID 51458739 (Internal validation), two water matrices were used in the study (pp. 9, 11, 18 of MRID 51458739). The ground water was obtained from a water monitoring study FTISN001 performed in Northern Iowa for isoxaflutole (IFT). The other water matrix was only described as "grocery store water" which was obtained from a local grocery store. No water characterization data was included in the study.
3 In the ILV, the surface water matrix (pond water) was collected by the testing facility from a local pond, but uncharacterized (pp. 6, 13; Appendix F, p. 86 of MRID 51458740).

I. Principle of the Method

An aliquot (*ca*. 5 to 10 mL) of the water samples were transferred to a labeled scintillation vial (pp. 7-10 of MRID 51458738; p. 12 of MRID 51458739). From that 5-10 mL aliquot, 1.0-mL aliquots were transferred into an HPLC vial then fortified (0.125 mL of a 0.0004 µg/mL or 0.004 µg/mL mixed fortification solution). Mixed internal standard (0.125 mL of a 0.004 µg/mL solution) was added with mixing then the sample was taken for LC-MS/MS analysis.

Samples were analyzed for diflufenican (AE F088657), AE 0542291 (phenoxynicotinamide), and AE B107137 (diflufenican acid) using a Shimadzu 20ADXR HPLC coupled with a AB Sciex Triple Quad 5500 LC/MS/MS operated in electrospray (ESI) interface and positive ionization mode with multiple reaction monitoring (MRM; pp. 12-13; Appendix 3, pp. 84-86 of MRID 51458739). The following LC conditions were used: Phenomenex Luna C18 (2)-HST 100A column (2.0 x 50 mm, 2.5 µm; oven temperature 40°C), mobile phase of (A) water:methanol (9:1, v:v) with 10mM ammonium formate and 120 µL formic acid per liter and (B) water:methanol (1:9, v:v) with 10mM ammonium formate and 120 µL formic acid per liter [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 100:0, 3.00-3.50 min. 0:100, 3:51-5.00 min. 100:0] and injection volume of 90 µL

(adjust as needed). MS temperature was 600°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.0→266.0 and *m/z* 395.0→246.0 for diflufenican (AE F088657), *m/z* 283.0→265.9 and *m/z* 283.0→246.0 for AE 0542291, and *m/z* 284.2→266.0 and *m/z* 284.2→238.0 for AE B107137. Reported retention times were *ca*. 4.0, 3.4, and 3.2 minutes for diflufenican (AE F088657), AE 0542291, and AE B107137, respectively. Two ion pair transitions were monitored for each internal standard (quantitation and confirmation, respectively): *m/z* 398.0→268.0 and *m/z* 398.0→248.0 for diflufenican-d_3 (AE F088657-d_3), *m/z* 286.0→267.9 and *m/z* 286.0→248.0 for AE 0542291-d_3, and *m/z* 287.2→268.0 and *m/z* 287.2→240.0 for AE B107137-d_3. All LC/MS/MS parameters in MRID 51458738 matched those in MRID 51458739; however, many of the mass transition ions were specified to the hundredths and thousandths place (pp. 6, 11-12 of MRID 51458738).

The ILV performed the ECM method (Bayer Method No. DC-004-W19-01) as written with insignificant modifications to the analytical parameters (pp. 12-18; Appendix F, pp. 86-88 of MRID 51458740). Samples were analyzed for diflufenican (AE F088657), AE 0542291 (phenoxynicotinamide), and AE B107137 (diflufenican acid) using Shimadzu LC-30AD HPLC coupled with an Sciex API 6500 Mass Spectrometer (pp. 16-17). The LC-MS/MS parameters were the same as those of the ECM, with the exception of some minor MS parameter modifications. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.2→266.000 and *m/z* 395.2→246.000 for diflufenican (AE F088657), *m/z* 283.1→266.000 and *m/z* 283.1→246.000 for AE 0542291, and *m/z* 284.1→266.000 and *m/z* 284.1→238.000 for AE B107137. These ion transitions were similar to those of ECM 1 and ECM 2. Reported retention times were *ca*. 3.20, 2.51, and 2.28 minutes for diflufenican (AE F088657), AE 0542291, and AE B107137, respectively. Two ion pair transitions were monitored for each internal standard (quantitation and confirmation, respectively): *m/z* 398.1→268.000 and *m/z* 398.1→248.000 for diflufenican-d_3 (AE F088657-d_3), *m/z* 286.2→268.000 and *m/z* 286.2→220.000 for AE 0542291-d_3, and *m/z* 287.1→268.000 and *m/z* 287.1→240.000 for AE B107137-d_3. These ion transitions were similar to those of ECM 1 and ECM 2, except for the confirmation ion transition for AE 0542291-d_3. The ILV modifications did not warrant an updated ECM.

The Limit of Quantification (LOQ) for diflufenican (AE F088657) and its degradates AE 0542291(phenoxynicotinamide) and AE B107137 (diflufenican acid) in water was 0.05 ng/mL in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458738; pp. 9, 15-16 of MRID 51458739; pp. 6, 18 of MRID 51458740). In the ILV, the Limit of Detection (LOD) for diflufenican, AE 0542291, and AE B107137 was reported as 0.02 ng/mL, equivalent to the lowest calibration standard (p. 15 of MRID 51458740). In the ECM 1 and ECM 2, the LOD for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 (p. 16; Table 7, p. 20 of MRID 51458739). The MDL for water (combined matrix data) was determined to be 0.004290 ng/mL, 0.003097 ng/mL, and 0.005781 ng/mL for diflufenican, AE 0542291, and AE B107137, respectively. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM 1 (MRID 51458738): MRID 51458738 was a method only study report. No performance data was submitted in this study.

ECM 2 (MRID 51458739): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) at fortification levels of 0.05 ng/mL (LOQ) and 0.5 ng/mL (10×LOQ) in two water matrices and one water matrix (Tables 1-6, pp. 19-20). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The ECM 2 MRID 51458739 (Internal validation), two water matrices were used in the study (pp. 9, 11, 18 of MRID 51458739). The ground water was obtained from a water monitoring study FTISN001 performed in Northern Iowa for isoxaflutole (IFT). The other water matrix was only described as "grocery store water" which was obtained from a local grocery store. No water characterization data was included in the study.

ILV (MRID 51458740): Mean recoveries and RSDs were within guidelines for analysis of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) at fortification levels of 0.05 ng/mL (LOQ) and 0.5 ng/mL (10×LOQ) in one water matrix (Table 1, p. 20). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses, except for the LOQ analysis of AE B107137. The surface water matrix (pond water) was collected by the testing facility from a local pond, but uncharacterized (pp. 6, 13; Appendix F, p. 86). The number of ILV trials required to validate the method (Bayer Method No. DC-004-W19-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for water was not reported; however, it was assumed that the ILV validated the method in the first trial as written with insignificant modifications to the analytical parameters (pp. 12-18; Appendix F, pp. 86-88). The ILV modifications did not warrant an updated ECM.

Table 2. Initial Validation Method Recoveries for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in Water[1,2,3]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water (Iowa)					
	Quantitation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	7	70-81	77	3.7	4.8
	0.5	5	75-79	76	1.6	2.1
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	7	72-80	76	2.7	3.5
	0.5	5	75-80	77	1.8	2.3
AE B107137 (Diflufenican acid)	0.05 (LOQ)	7	72-85	78	4.5	5.8
	0.5	5	74-76	75	1.2	1.6
	Confirmation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	7	70-78	73	2.9	4.0
	0.5	5	73-77	75	1.8	2.5
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	7	71-81	77	4.3	5.6
	0.5	5	74-77	75	1.3	1.7
AE B107137 (Diflufenican acid)	0.05 (LOQ)	7	77-90	81	5.1	6.3
	0.5	5	73-77	76	1.5	2.1
	"Grocery Store" Water					
	Quantitation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	7	72-80	75	2.7	3.6
	0.5	5	73-76	75	1.2	1.6
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	7	73-78	75	1.9	2.5
	0.5	5	74-78	76	1.6	2.1
AE B107137 (Diflufenican acid)	0.05 (LOQ)	7	75-86	80	3.9	4.8
	0.5	5	70-76	74	2.1	2.8
	Confirmation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	7	70-78	74	2.6	3.6
	0.5	5	73-77	75	1.3	1.7
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	7	74-82	77	2.6	3.4
	0.5	5	73-76	74	1.3	1.7
AE B107137 (Diflufenican acid)	0.05 (LOQ)	7	72-88	78	5.5	7.0
	0.5	5	72-78	74	2.4	3.2

Data (uncorrected recovery results; p. 14; Appendix 1, pp. 25-48; Appendix 4, pp. 87-88 of MRID 51458739) were obtained from Tables 1-6, pp. 19-20 of MRID 51458739.

1 ECM 1 MRID 51458738 was a method only study report. No performance data was submitted in this study.

2 In the ECM 2 MRID 51458739 (Internal validation), two water matrices were used in the study (pp. 9, 11, 18 of MRID 51458739). The ground water was obtained from a water monitoring study FTISN001 performed in Northern Iowa for isoxaflutole (IFT). The other water matrix was only described as "grocery store water" which was obtained from a local grocery store. No water characterization data was included in the study.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.0→266.0 and *m/z* 395.0→246.0 for diflufenican (AE F088657), *m/z* 283.0→265.9 and *m/z* 283.0→246.0 for AE 0542291, and *m/z* 284.2→266.0 and *m/z* 284.2→238.0 for AE B107137.

Table 3. Independent Validation Method Recoveries for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in Water[1,2]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface (Pond) Water					
	Quantitation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	5	97.9-112	106	5.4	5.2
	0.5	5	104-113	110	3.4	3.1
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	5	88.6-119	108	11.5	10.6
	0.5	5	106-110	108	1.8	1.7
AE B107137 (Diflufenican acid)	0.05 (LOQ)	5	75.9-94.9	84.6	7.2	8.6
	0.5	5	107-115	110	3.0	2.7
	Confirmation ion transition					
Diflufenican (AE F088657)	0.05 (LOQ)	5	88.1-113	102	9.9	9.6
	0.5	5	104-111	107	2.6	2.5
AE 0542291 (Phenoxynicotinamide)	0.05 (LOQ)	5	95.0-116	106	7.4	7.0
	0.5	5	99.6-108	103	3.8	3.7
AE B107137 (Diflufenican acid)	0.05 (LOQ)	5	96.0-115	109	9.7	8.9
	0.5	5	97.3-107	102	3.6	3.5

Data (uncorrected recovery results; p. 17) were obtained from Table 1, p. 20 of MRID 51458740.

1 The surface water matrix (pond water) was collected by the testing facility from a local pond, but uncharacterized (pp. 6, 13; Appendix F, p. 86 of MRID 51458740).

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.2→266.000 and *m/z* 395.2→246.000 for diflufenican (AE F088657), *m/z* 283.1→266.000 and *m/z* 283.1→246.000 for AE 0542291, and *m/z* 284.1→266.000 and *m/z* 284.1→238.000 for AE B107137.

III. Method Characteristics

The LOQ for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in water/water was 0.05 ng/mL in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458738; pp. 9, 15-16 of MRID 51458739; pp. 6, 18 of MRID 51458740). In the ECM 2, the LOQ was defined as the lowest fortification level which yields a mean recovery between 70 and 120% with a relative standard deviation of ≤20%, as well as having the blank values below 30%. No justification for the LOQ was reported in ECM 1 and ILV. No LOQ calculations were reported in the ECM 1, ECM 2, or ILV. In the ILV, the Limit of Detection (LOD) for diflufenican, AE 0542291, and AE B107137 was reported as 0.02 ng/mL, equivalent to the lowest calibration standard (p. 15 of MRID 51458740). In the ECM 1 and ECM 2, the LOD for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 (p. 16; Table 7, p. 20 of MRID 51458739):

$MDL = (SD \times t_{0.99})$

Where, $t_{0.99}$ is the one-tailed t statistic for n -1 and SD is the standard deviation of the analyte recovery measurements at the target LOQ. For the water MDL (combined matrix data), n = 14 and $t_{0.99}$ is 2.650.

This MDL calculation was in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016); however, it may be preferable to calculate the MDLs for each water separately.

In ECM 2, the MDL for water (combined matrix data) was determined to be 0.004290 ng/mL, 0.003097 ng/mL, and 0.005781 ng/mL for diflufenican, AE 0542291, and AE B107137, respectively.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics in Water

		Diflufenican (AE F088657)	AE 0542291 (Phenoxynicotinamide)	AE B107137 (Diflufenican acid)
Limit of Quantitation (LOQ)*	ECM 1[1]	0.05 ng/mL		
	ECM 2[2]			
	ILV			
Limit of Detection (LOD)	ECM 1	**Not reported**		
	ECM 2	**Not reported**		
		MDL (calc) = 0.004290 ng/mL (combined waters)	MDL (calc) = 0.003097 ng/mL (combined waters)	MDL (calc) = 0.005781 ng/mL (combined waters)
	ILV	0.02 ng/mL		
Linearity (calibration curve r and concentration range)	ECM 1[3,5]	r = 0.9994 (Q)	r = 0.9990 (Q)	r = 0.9990 (Q)
	ECM 2[4,5]	r = 0.9982 (Q, GW) r = 0.9978 (C, GW) r = 0.9968 (Q, GS) r = 0.9967 (C, GS)	r = 0.9972 (Q, GW) r = 0.9969 (C, GW) r = 0.9981 (Q, GS) r = 0.9975 (C, GS)	r = 0.9976 (Q, GW) r = 0.9979 (C, GW) r = 0.9975 (Q, GS) r = 0.9973 (C, GS)
	ILV[5]	r = 0.9993 (Q, SW)	r = 0.9978 (Q, SW)	r = 0.9987 (Q, SW)
	Range	0.02-2 ng/mL		
Repeatable	ECM 1[1]	No performance data submitted. Method only.		
	ECM 2[6]	Yes at LOQ (0.05 ng/mL) and 10×LOQ (0.5 ng/mL) (two uncharacterized water matrices)		
	ILV[7,8]	Yes at LOQ (0.05 ng/mL) and 10×LOQ (0.5 ng/mL) (one uncharacterized water matrix)		
Reproducible		Yes for 0.05 ng/mL (LLMV)* and 0.5 ng/mL in water matrices		
Specific	ECM 1	Yes, no matrix interferences were observed, but **no** representative control chromatograms were provided (matrix undefined). For AE B107137, some nearby baseline noise was observed at the LOQ.		
	ECM 2	Yes, matrix interferences were <10% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Baseline noise was noted around the LOQ analyte peak.
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline noise was noted around the analyte peak interfered with peak attenuation and integration.[9]	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline noise and contaminant (RT *ca*. 2.75 min.) were noted around the LOQ analyte peak which interfered with peak attenuation and integration. [10]	**No**, matrix interferences were *ca*. 15% of the LOQ (based on peak area); however, significant baseline noise was noted around the LOQ analyte peak and LOQ analyte peak response was only slightly greater than matrix contaminants. [11]
		Only representative chromatograms of the quantitation ion analysis were provided.[12]		

Data were obtained from p. 6 (LOQ/LOD); p. 9; Appendix 2, pp. 16-18 (calibration curves); Appendix 3, pp. 19-21 (chromatograms) of MRID 51458738; pp. 9, 15-16; Table 7, p. 20 (LOQ/LOD); Tables 1-6, pp. 19-20 (recovery results); p. 14; Appendix 1, pp. 25-36 (calibration curves); Appendix 2, pp. 55-83 (chromatograms) of MRID 51458739; pp. 6, 15, 18 (LOQ/LOD); Table 1, p. 20 (recovery results); p. 15; Appendix B, Figures B-1 to B-3, pp. 31-33 (calibration curves); Appendix C, Figures C-1 to C-30, pp. 36-65 (chromatograms) of MRID 51458740; DER Excel

Attachment. Q = quantitation ion transition; C = confirmation ion transition. GW = Ground water; GS = Grocery store water: SW = Surface water.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 ECM 1 MRID 51458738 was a method only study report. No performance data was submitted in this study.

2 ECM 2 MRID 51458739 (Internal validation) contained the method summary and performance data.

3 Only one set of calibration curves were provided, the quantitation ion transition. The matrix used was not specified.

4 ECM 2 correlation coefficients (r) were reviewer-calculated based on r^2 values reported in the study report (Appendix 1, pp. 25-36 of MRID 51458739; DER Excel Attachment).

5 In the ECM 1, ECM 2, and ILV, solvent-based calibration standards were used for all analyses (p. 9 of MRID 51458738; pp. 12, 14 of MRID 51458739; p. 15 of MRID 51458740). Matrix effects were not studied in ECM 1, ECM 2, and ILV, since the addition of the deuterated standard was used for analysis.

6 In the ECM 2 MRID 51458739 (Internal validation), two water matrices were used in the study (pp. 9, 11, 18 of MRID 51458739). The ground water was obtained from a water monitoring study FTISN001 performed in Northern Iowa for isoxaflutole (IFT). The other water matrix was only described as "grocery store water" which was obtained from a local grocery store. No water characterization data was included in the study.

7 In the ILV, the surface water matrix (pond water) was collected by the testing facility from a local pond, but uncharacterized (pp. 6, 13; Appendix F, p. 86 of MRID 51458740).

8 The number of ILV trials required to validate the method (Bayer Method No. DC-004-W19-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for water was not reported; however, it was assumed that the ILV validated the method in the first trial as written with insignificant modifications to the analytical parameters (pp. 12-18; Appendix F, pp. 86-88 of MRID 51458740). The ILV modifications did not warrant an updated ECM.

9 Based on Appendix C, Figures C-9 to C-10, pp. 44-45 of MRID 51458740.

10 Based on Appendix C, Figure C-19, p. 54 of MRID 51458740.

11 Based on Appendix C, Figure C-29, p. 64 of MRID 51458740.

12 Deviations in the confirmation ion analysis do not affect the specificity of the method since a confirmation method is not usually required when GC/MS and/or LC/MS is used as the primary method to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. CDM/CSS-Dynamac JV personnel received the following directive from the Environmental Fate and Effects Division regarding the preparation of the DER for Bayer CropScience Method No. DC-002-S18-01 for soil/sediment with MRIDs 51458735 & 51458736 & 51458737 (email dated 09/17/2021):

   "The bigger issue is that MRID 51458735 should not be cited in the DER if it is redundant and less complete than MRID 51458736, which contains the validation results. There's no requirement that we review every MRID submitted to EPA. We should review the relevant analytical methods that have paired ECM & ILV reports, not every submitted ECM or ILV report."

   This directive also applied to the preparation of this DER which included MRIDs 51458738 & 51458739 & 51458740. However, MRID 51458738 was included in this DER for the following reason:

   > ECM 2 MRID 51458739 (Internal validation) contained only a method summary, while ECM 1 MRID 51458738 contained the full method written in a step-wise manner (p. 10 of MRID 51458738; p. 12 of MRID 51458739). In OCSPP guideline 850.6100 states that "The ECM should be clearly written with complete analytical methods that describe the exact procedure, materials, and equipment in sufficient detail to be used by laboratory chemists to review the ECM and its associated ILV results".
   >
   > OCSPP guideline 850.6100 could not have been satisfied using only ECM 2 MRID 51458739 since the ECM method (Bayer Method No. DC-004-W19-01) was not completely reproduced in ECM 2 MRID 51458739. Method details such as volume of mixed internal standard solution to add to samples and calibration standard preparation were not included in ECM 2 MRID 51458739. These details were found in ECM 1 MRID 51458738.

2. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (p. 6 of MRID 51458738; pp. 9, 15-16 of MRID 51458739; pp. 6, 18 of MRID 51458740). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM 2, the LLMV was equivalent to the ECM reported method LOQ for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in the tested water matrices (0.05 ng/mL).

3. The specificity of the method for AE B107137 was not supported by ILV representative chromatograms due to significant baseline noise around the LOQ analyte peak and the fact that the LOQ analyte peak response was only slightly greater than matrix contaminants (Appendix C, Figure C-29, p. 64 of MRID 51458740). The reviewer noted that performance data between the quantitation and confirmation analyses was not comparable for the LOQ analysis of AE B107137. Only representative chromatograms of the quantitation ion analysis were provided for review in the ILV study report.

4. Only one water was used in the ILV, and the water was not characterized (pp. 6, 13; Appendix F, p. 86 of MRID 51458740). The ILV water matrix was only described as pond water from a local pond in the ILV communications to the ILV Study Sponsor. Additionally, the internal validation (ECM 2) validated the method using two water matrices. Even though a certain number of water matrices is not specified in the OCSPP guidelines, the ILV should provide a validation which is as rigorous or more rigorous than the internal validation.

   In ECM 2 MRID 51458739 (Internal validation), two uncharacterized water matrices (ground water and "grocery store water") were used in the study (pp. 9, 11, 18 of MRID 51458739).

5. The number of ILV trials required to validate the method (Bayer Method No. DC-004-W19-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for water was not reported. The reviewer assumed that the ILV validated the method in the first trial since only insignificant modifications of the analytical parameters were reported by the ILV.

6. The ILV (MRID 51458740) was conducted independently of the internal validation (ECM 2 MRID 51458739) and method authors (ECM 1 MRID 51458739; pp. 1, 6 of MRID 51458739; Appendix F, pp. 86-88 of MRID 51458740). The communications between the ILV study author (Penny Miner, Frontage Laboratories) and ILV Study Monitor (Chung Lam, Bayer CropScience) were provided as the email correspondence (pp. 1, 4; Appendix F, pp. 86-88 of MRID 51458740). The only other personnel involved in the email communications was Derek Netzband (Bayer CropScience) who signed the Authenticity Statement for Chung Lam. Reported communications included: ILV validation results and discussion of which type of water matrix to use for the ILV validation. The only technical advice which was given by the ILV Study Sponsor was to not use "tap water or any 'purified' water – HPLC, distilled etc." (Appendix F, p. 86). The reviewer viewed this communication as protocol questions and not direct technical solutions to ILV issues.

   However, the reviewer noted that Bayer Method No. DC-004-W19-01 may need to be updated with precautions against the use of tap or 'purified' water.

7. The reviewer noted that interferences to LOQ analyte peak attenuation and integration were observed in ILV representative chromatograms of diflufenican and AE 0542291 [baseline contaminant (RT *ca*. 2.75 min.) and/or baseline noise; Appendix C, Figures C-9 to C-10, pp. 44-45 and Appendix C, Figure C-19, p. 54 of MRID 51458740].

8. The determinations of the LOQ and LOD in the ECM 1, ECM 2, and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in water was 0.05 ng/mL in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458738; pp. 9, 15-16 of MRID 51458739; pp. 6, 18 of MRID 51458740). In the ILV, the Limit of Detection (LOD) for diflufenican, AE 0542291, and AE B107137 was reported as 0.02 ng/mL, equivalent to the lowest calibration standard (p. 15 of MRID 51458740). In the ECM 1 and ECM 2, the LOD for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 using the following equation (p. 16; Table 7, p. 20 of MRID 51458739): MDL = (SD × $t_{0.99}$),

where, $t_{0.99}$ is the one-tailed t statistic for n -1 and SD is the standard deviation of the analyte recovery measurements at the target LOQ. For the water (combined matrix data) MDL, n = 14 and $t_{0.99}$ is 2.650. This MDL calculation was in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016); however, it may be preferable to calculate the MDLs for each water separately.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

9. The justification for selection of diflufenican (AE F088657), and degradates diflufenican-amide (AE 0542291; DFF-amide) and diflufenican-acid (AE B107137; DFF-acid) for water, water, and water methods was described in an accompanying supplemental MRID 51458658 (Shrestha, S. 2021. Justification for Selection of Analytes for Water, Water Methods and Terrestrial Field Dissipation Studies. Bayer CropScience Report No. US0819. Bayer CropScience LP, St. Louis, Missouri). Residue definition for environmental chemistry methods was determined by assessing the maximum occurrence of each degradate that occurred above 10% of applied in environmental fate laboratory studies, including aerobic water metabolism, aerobic and anaerobic aquatic metabolism, and water and aqueous photolysis. DFF-amide was only detected at >10% of the applied in aerobic water metabolism studies. DFF-acid was detected in at >10% of the applied in aerobic water metabolism and aerobic and anaerobic aquatic metabolism studies. No major degradates were detected in water and aqueous photolysis studies. The toxicological significance of any diflufenican degradate was not reported.

10. The stabilities of the final sample extract and calibration solutions were studied in the ECM 2 (pp. 16-17; Tables 8-9, pp. 21-23; Appendix 1, pp. 37-48 of MRID 51458739). The 10×LOQ ground water samples were determined to be stable for up to 6 days at 1 to 6°C storage. The 2 ng/mL calibration standards were determined to be stable for up to 6 days with frozen storage (≤-18°C) or refrigerated storage (1 to 6°C).

11. The total time required to complete one sample set was reported as one 8-hour day in the ILV (p. 15 of MRID 51458740). The total time required to complete one set of fifteen samples was reported in the ECM as less than one calendar day with LC/MS/MS requiring *ca.* 4 hours (p. 17 of MRID 51458739).

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.

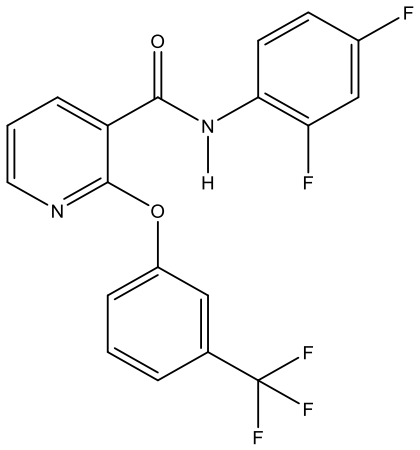
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347**,** and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

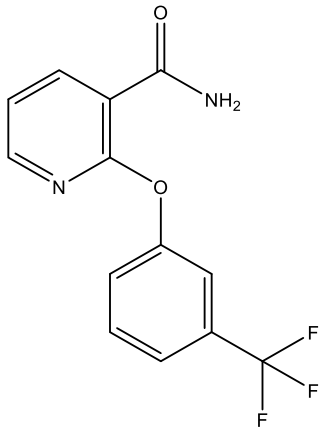
Diflufenican (AE F088657; M&B 38544)

IUPAC Name: 2',4'-Difluoro-2-[(α,α,α-trifluoro-m-tolyl)oxy]nicotinanilide
CAS Name: N-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]- 3-pyridinecarboxamide
CAS Number: 83164-33-4
SMILES String: n1c(Oc2cc(C(F)(F)F)ccc2)c(C(=O)Nc3c(F)cc(F)cc3)ccc1



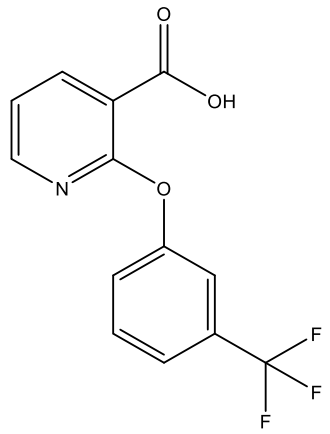
AE 0542291 (M&B 43625; Diflufenican-amide; DFF-amide; Phenoxynicotinamide)

IUPAC Name: 2-(3-Trifluoromethyl)phenoxynicotinamide
CAS Name: 2-(3-Trifluoromethyl)phenoxy-3-pyridinecarboxamide
CAS Number: Not reported
SMILES String: NC(C1=C(OC2=CC=CC(C(F)(F)F)=C2)N=CC=C1)=O



AE B107137 (Diflufenican-acid; M&B 38181; DFF-acid)

IUPAC Name: 2-[3-(Trifluoromethyl)phenoxy]nicotinic acid
CAS Name: 2-(3-Trifluoromethyl)phenoxy-3-pyridinecarboxylic acid
CAS Number: 36701-89-0
SMILES String: OC(C1=CC=CN=C1OC2=CC=CC(C(F)(F)F)=C2)=O



Diflufenican-d_3

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CAS Name: Not reported
CAS Number: Not reported
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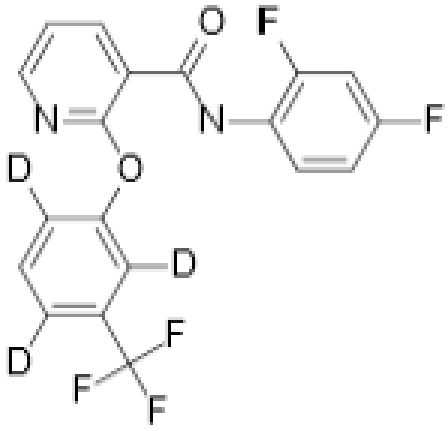

AE 0542291-d_3

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CAS Name: Not reported
CAS Number: Not reported
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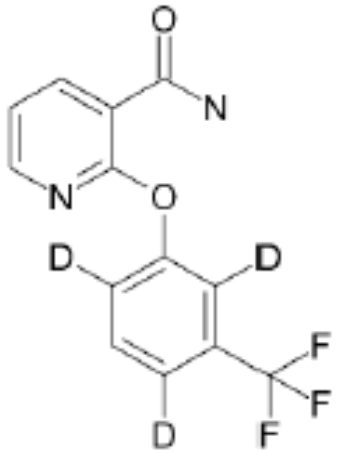

Diflufenican acid-d_3 (AE B107137- d_3)

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found