Title

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 --

Test Substance

AE F088657

Data Requirement

US EPA Residue Chemistry Test Guidelines
OCSPP 860.1340 Residue Analytical Method
US EPA Ecological Effects Test Guidelines
OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation
PMRA: DACO 7.2.2 Residue Analytical Method
Guidance document on residue analytical methods; SANCO/825/00 rev. 7

Completion Date

2019-10-14

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Study ID

RADC0097

Activity ID

RADC0097

GENERAL INFORMATION

Study Title:	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
Sponsor:	Bayer CropScience LP 800 N. Lindbergh Blvd. St. Louis, MO 63167
Testing Facility Management:	Derek Netzband Manager, Residue Chemistry Bayer CropScience LP
Study Director:	Mengyuan Wang Bayer CropScience LP
Bayer CropScience Study No.:	RADC0097
Test Items/ Reference Standards:	AE F088657, AE 0542291 and AE B107137
Test Systems:	Ground Water and Grocery Store Water
Study Initiation Date: Experimental Start Date: Experimental Completion Date	13 March 2019 15 March 2019 21 March 2019
Personnel Involved In Study:	Mengyuan Wang

1.0 SUMMARY

The purpose of this study was to validate Bayer CropScience analytical method DC-004-W19-01 entitled “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”, as written [4] dated 2/20/2019.

The method was validated using a sub-sample of bulk untreated ground water sample from Iowa collected for study FTISN001 [5] and grocery store water obtained from a local grocery store. These samples were stored in refrigerator prior to fortification. After fortification, the water extracts were stored in a refrigerator until they were analyzed using analytical method DC-004-W19-01.

In brief, the method used for analysis was as follows:

An aliquot of water was fortified with a standard solution of AE F088657 (diflufenican), AE 0542291 and AE B107137. Possible matrix effects of AE F088657, AE 0542291 and AE B107137 were eliminated by using an internal standard solution of isotopically labeled reference items. Identification and quantitation of the active substance was performed by high performance liquid chromatography using tandem MS/MS detection (LC-MS/MS) in the Multiple Reaction Monitoring (MRM) mode using the following MRM transitions:

Analyte	Quantitation MRM	Confirmation MRM
AE F088657	395.0 → 266.0	395.0 → 246.0
AE B107137	284.2 → 266.0	284.2 → 238.0
AE 0542291	283.0 → 265.9	283.0 → 246.0

Samples were fortified at 0.05 ng/mL (ppb) (LOQ) and 0.5 ng/mL (10x LOQ) for AE F088657, AE 0542291 and AE B107137 in both ground water (GW-water) and grocery store water (GS-water) samples.

The correlation between the injected amount of substance and the detector response was linear for the calibration standards ranging from 0.02 ng/mL to 2 ng/mL, with correlation coefficients of > 0.99. The method meets all guideline criteria to determine residues of AE F088657, AE 0542291 and AE B107137 in water.

2.0 INTRODUCTION

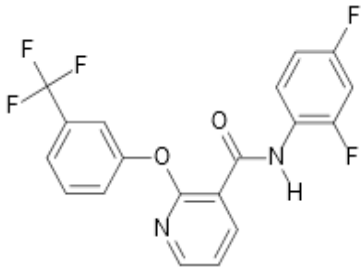
The purpose of this study was to validate the analytical methods DC-004-W19-01 for AE F088657, AE 0542291 and AE B107137 in water. This study was conducted to satisfy guideline requirements described in the US EPA guidance documents Ecological Effects Test Guidelines OCSPP 850.6100 and Residue Chemistry Test Guidelines OCSPP 860.1340, PMRA DACO 7.2.2 Residue Analytical Method and Guidance document on residue analytical methods; SANCO/825/00 rev. 7. In addition, this study will be conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

3.0 MATERIALS AND METHODS

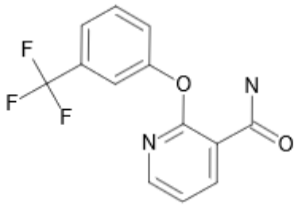
3.1 TEST ITEMS/REFERENCE SUBSTANCES

Only sufficiently characterized and certified substances were used as reference items.

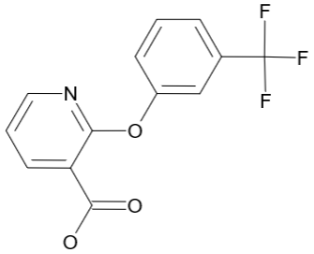
Code Name:	AE F088657
Synonym:	Diflufenican
CAS Name:	*N*-(2,4-Difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide
Molecular Formula:	$C_{19}H_{11}F_5N_2O_2$
Molecular Weight:	394.29 g/mol
CAS No.:	83164-33-4
ID No.:	K-1412
Purity:	99.8%
Expiration Date:	4/21/2024



Code Name:	AE 0542291
CAS Name:	2-(3-Trifluoromethoxy)phenoxy-3-pyridinecarboxamide
Molecular Formula:	$C_{13}H_9F_3N_2O_2$
Molecular Weight:	282.22 g/mol
ID No.:	K-2230
Purity:	99.0%
Expiration Date:	7/18/2022

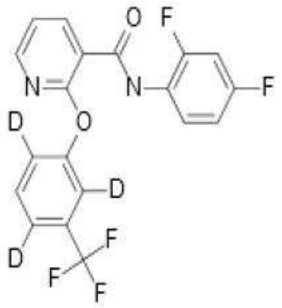


Code Name:	AE B107137
Synonym:	MB 38181
CAS Name:	2-[3-(Trifluoromethyl)phenoxy]-3-pyridinecarboxylic acid
Molecular Formula:	$C_{13}H_8F_3NO_3$
Molecular Weight:	283.20 g/mol
CAS No.:	36701-89-0
ID No.:	K-2238
Purity:	98.9%
Expiration Date:	7/12/2022

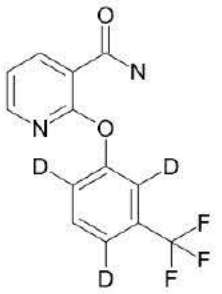


3.2 INTERNAL STANDARDS

Code Name:	AE F088657-d_3
Molecular Formula:	$C_{19}H_8D_3F_5N_2O_2$
Molecular Weight:	397.32 g/mol
ID No.:	K-2255
Purity:	100%
Expiration Date:	6/20/2027



Code Name:	AE 0542291-d_3
Molecular Formula:	$C_{13}D_3H_6F_3N_2O_2$
Molecular Weight:	285.24 g/mol
ID No.:	K-2295
Purity:	100%
Expiration Date:	6/20/2027



Code Name:	AE B107137-d_3
Molecular Formula:	$C_{13}D_3H_5F_3NO_3$
Molecular Weight:	286.22 g/mol
ID No.:	K-2257
Purity:	100%
Expiration Date:	6/20/2027

Characterization data for the reference substances (analytical standards) and internal standards are maintained by Bayer CropScience.

The reference substances (analytical standards) and internal standard used in this study were procured from Bayer CropScience and stored as directed on "Analytical Standards Chain of Custody" and "Certificate of Analysis" documents. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.3 TEST SYSTEMS

An analytical method DC-004-W19-01 was evaluated in two different waters, both of which originated in the United States. One sample was collected in water monitoring study FTISN001 [5] performed in the United States in the state of Iowa (IA). The other was grocery store water obtained from a local grocery store.

3.4 SAMPLE PREPARATION AND EXTRACTION

Bayer analytical method DC-004-W19-01 was used for the analysis of AE F088657, AE 0542291 and AE B107137. The preparation of the water residues was as follows:

Water samples (1 mL) were aliquoted and fortified with a fortification standard solution. Possible matrix effects of AE F088657, AE 0542291 and AE B107137 were eliminated by using an internal standard solution of isotopically labeled reference items. An aliquot was transferred to a LC vial and then analyzed by LC/MS/MS.

Two product ions per analyte were selected for monitoring; one ion mass transition served as quantitation and the other ion mass transition was used for confirmation. The mass transitions for both the quantitation as well as the confirmation for all substances are presented below:

Analyte	Quantitation MRM[a]	Confirmation MRM[a]
AE F088657	395.0 → 266.0	395.0 → 246.0
AE B107137	284.2 → 266.0	284.2 → 238.0
AE 0542291	283.0 → 265.9	283.0 → 246.0

[a] All masses reported in amu

3.5 INSTRUMENTATION

Standard Method for all Analytes

The HPLC conditions employed were as follows:

HPLC Systems: Shimadzu 20ADXR HPLC Pumps
Shimadzu CBM-20A Controller
Shimadzu CTO-20A Column Oven
PAL DHR Autosampler

Mobile Phase A: 9:1 water:methanol (v/v) with 10 mM ammonium formate and 120 µL per 1000 mL formic acid

Mobile Phase B: 1:9 water:methanol (v/v) with 10 mM ammonium formate and 120 µL per 1000 mL formic acid

Oven: 40 ºC

Pre column (optional): none

HPLC Column: Phenomenex Luna C18(2)-HST 100A 50 mm x 2.0 mm 2.5 µm particle (Part # 00B-4446-B0)

Injection Volume: 90 µL (adjust as needed)

Gradient:

Time (min)	Mobile Phase B%	Flow rate µL/min
0.50	0	500
3.00	100	500
3.50	100	500
3.51	0	500
5.00	Stop Run	500

Retention time:

Analyte	Approx Retention Time (min)
AE B107137	3.2
AE 0542291	3.4
AE F088657	4.0

The MS/MS conditions employed were as follows:

MS System: AB Sciex Triple Quad 5500 LC/MS/MS System with Analyst Software (ver. 1.6.2) and Chronos Software (ver. 4.7.2.0)

Interface: Electrospray (ESI)
Ionization Mode: Positive (+)
Scan Type: MRM

Ion Spray Voltage (IS): 5500 V
Temperature (TEM): 600 ºC
Ion Source Gas 1(GS1): 70
Ion Source Gas 2(GS2): 80
Curtain Gas(CUR): 35
Collision Gas (CAD): 9
Resolution Q1: Unit
Resolution Q3: Unit

Analyte Name[a]	Q1 Mass (amu)	Q3 Mass (amu)	Time (msec)	CE	CXP	DP	EP
AE B107137-Q	284.2	266.0	35	27	20	65	10
AE B107137-C	284.2	238.0	35	37	20	65	10
AE B107137 IS-Q	287.2	268.0	35	27	20	65	10
AE B107137 IS-C	287.2	240.0	35	37	20	65	10
AE 0542291-Q	283.0	265.9	35	27	20	65	10
AE 0542291-C	283.0	246.0	35	33	20	65	10
AE 0542291 IS-Q	286.0	267.9	35	27	20	65	10
AE 0542291 IS-C	286.0	248.0	35	33	20	65	10
AE F088657-Q	395.0	266.0	35	23	20	65	10
AE F088657-C	395.0	246.0	35	57	20	65	10
AE F088657-IS-Q	398.0	268.0	35	23	20	65	10
AE F088657-IS-C	398.0	248.0	35	57	20	65	10

[a] Q is MRM transition for quantitation MRM and C for the confirmatory MRM transition

Example LC/MS/MS conditions are presented in Appendix 3.

3.6 CALCULATIONS

The example calculation displayed below was used by the laboratory performing the independent laboratory validation. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Residue concentrations were determined using calibration curves which were generated after each analysis using ABSciex quantitation software Analyst (Version 1.6.2) using linear regression with 1/x weighting.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area: isotopic peak area ratio

After regression coefficients were calculated, the residue in ng/mL was determined using the following equation:

$$\text{Residue found (ng/mL)} = \frac{(Y-B)}{M}$$

Analyst software was used to calculate the amount of AE F088657, AE 0542291 and AE B107137 in ng/mL for each sample and the percent recovery for the spiked samples.

The percent recovery was calculated as follows:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where:
R = ng/mL of target analyte found in fortified (recovery) sample
S = ng/mL of target analyte found in control sample, real or apparent
T = theoretical ng/mL in fortified sample

4.8 TIME REQUIREMENTS

In less than one calendar day, Mengyuan Wang, completed one sample set which ran on LC-MS/MS approximately 4 hours.

5.0 CONCLUSION

Bayer CropScience in the U.S. successfully independently validated Bayer CropScience analytical methods entitled “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” [4]. The method was validated on the first validation set.

The method was demonstrated to be suitable for the determination of all targeted analytes in two separate waters collected from sites in the United States. A LOQ of at least 0.05 ng/mL was demonstrated for each analyte in each matrix evaluated.

6.0 ARCHIVING

All raw data associated with this study are retained along with the protocol, protocol amendments and the final report under Notebook Number RADC0097 at Bayer CropScience, 700 Chesterfield Parkway West, Chesterfield, MO 63017.

7.0 REFERENCES

1. U.S. EPA Ecological Effects Test Guidelines OCSPP 850.6100 Data Reporting for Environmental Chemistry Methods, Public Draft, January 2012.

2. U.S. EPA Residue Chemistry Test Guidelines OCSPP 860.1340 Residue Analytical Method, August 1996.

3. U.S. Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Registry, Vol. 54, No. 158: pp. 34052-34074.

4. Wang, M., Analytical Method DC-004-W19-01 for the Determination of Residues of AE F088657 and its metabolites AE 0542291 and AE B107137 in Water Using LC/MS/MS, 2019.

5. Xu, T., Groundwater Monitoring Program in Northern Iowa for Isoxaflutole (IFT) Registration in Minnesota, FTISN001, 2017.

Appendix 3. Typical HPLC-MS/MS Parameters

File Information for Sample 19 (19DC-GS-LOQ-005) of DC031519-MW2.wiff

File Name:	DC031519-MW2.wiff
File Path:	C:\Analyst Data\Projects\AE F088657\RADC0097\Data\
Original Name:	DC031519-MW2.wiff
Software Version:	Analyst 1.6.2

Acquisition Info

Acquisition Method:	\DC-003-P18-01.dam
Acquisition Path:	D:\Analyst Data\Projects\AE F088657\Acquisition Methods\
First Sample Started:	Friday, March 15, 2019 4:39:55 PM
Last Sample Finished:	Friday, March 15, 2019 8:28:44 PM
Sample Acq Time:	Friday, March 15, 2019 6:41:07 PM
Sample Acq Duration:	5min0sec
Number of Scans:	0
Periods in File:	1
Batch Name:	\Chronos.dab
Batch Path:	D:\Analyst Data\Projects\AE F088657\RADC0097\Batch\D:\Analyst Data\Projects\AE F088657\RADC0097\Batch\
Submitted by:	AUSREC9911\sciex(sciex)
Logged-on User:	sciex
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Comment:	
Software Version:	Analyst 1.6.2
Set Name:	Set1
Sample Name	19DC-GS-LOQ-005
Sample ID	
Sample Comments:	
Autosampler Vial:	35
Rack Code:	0
Rack Position:	0
Plate Code:	Drawer 3:Slot1
Plate Position	0

Valco Valve	Diverter	
	Total Time (min)	Position
1	0.5	B
2	5.0	A

Shimadzu LC Method Properties

Shimadzu LC system Equlibration time = 0.00 min
Shimadzu LC Method Parameters

Pumps

Pump A Model: LC-20ADXR
Pump B Model: LC-20ADXR
Pump C Model: LC-20ADXR
Pumping Mode: Ternary Flow
Total Flow: 0.5000 mL/min
Pump B Conc: 0.0 %
Pump C Conc: 0.0 %

B Curve: 0
C Curve: 0
Pressure Range (Pump A/B/C): 0 - 9222 psi

Oven
Model: CTO-20A
Temperature Control: Enabled
Temperature: 40 deg. C
Max. Temperature: 85 deg. C

System Controller
Model: CBM-20A
Power: On

Pump A (FCV-11AL)
Port 1 Valve Position: A - 9:1 H2O:MeOH_10mm AF_FA
Port 2 Valve Position: A - 1:9 H2O:MeOH_10mm AF_FA
Port 3 Valve Position: B - 00B-4446-B0

Time Program

Time	Module	Events	Parameter
0.50	Pumps	Pump B Conc.	0
3.00	Pumps	Pump B Conc.	100
3.50	Pumps	Pump B Conc.	100
3.51	Pumps	Pump B Conc.	0
5.00	System Controller	Stop	

Quantitation Information:
Sample Type: QC
Dilution Factor: 1.000000

Period 1:
Scans in Period: 625
Relative Start Time: 0.00 msec
Experiments in Period: 1

Period 1 Experiment 1:
Scan Type: MRM (MRM)
Scheduled MRM: No
Polarity: Positive
Scan Mode: N/A
Ion Source: Turbo Spray
Resolution Q1: Unit
Resolution Q3: Unit
Intensity Thres.: 0.00 cps
Settling Time: 50.0000 msec
MR Pause: 5.0070 msec
MCA: No
Step Size: 0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
284.224	266.000	35.00	CE	27.00	27.00	AE B107137-Q
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
284.224	238.000	35.00	CE	37.00	37.00	AE B107137-C
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
287.224	268.000	35.00	CE	27.00	27.00	AE B107137-Q-IS
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
287.224	240.000	35.00	CE	37.00	37.00	AE B107137-C-IS
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
283.005	265.900	35.00	CE	27.00	27.00	AE 0542291-Q
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
283.005	246.000	35.00	CE	33.00	33.00	AE 0542291-C
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
286.005	267.900	35.00	CE	27.00	27.00	AE 0542291-Q-IS
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
286.005	248.000	35.00	CE	33.00	33.00	AE 0542291-C-IS
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
395.000	266.000	35.00	CE	23.00	23.00	AE F088657-Q
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
395.000	246.000	35.00	CE	57.00	57.00	AE F088657-C
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
398.000	268.000	35.00	CE	23.00	23.00	AE F088657-Q-IS
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
398.000	248.000	35.00	CE	57.00	57.00	AE F088657-C-IS

Parameter Table(Period 1 Experiment 1)

CUR:	35.00
CAD:	9.00
TEM:	600.00
GS1:	70.00
GS2:	80.00
IS:	5500.00
DP	65.00
EP	10.00
CXP	20.00

Appendix 4. Example Calculations

AE F088657 residues were quantified using internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the ratio of standard peak/internal standard peak areas versus the standard concentrations in ng/mL using Analyst Software, a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients a and b, respectively called slope and intercept, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area/isotopic peak area ratio

The example shown below is for the calculation of AE F088657 metabolite, AE B107137 residue for sample 19DC-GS-10X-005 which was fortified with 0.5 ng/mL of AE B107137. This chromatogram is presented in Appendix 2, page 82.

After regression coefficients were calculated, the residue in ng/mL was determined. The ng/mL of AE B107137 in water was calculated using the following equation,

$$\text{AE B107137 found (ng/mL)} = \frac{(Y-B)}{M}$$

V_1	Native Peak Area	IS Peak Area	Y	M	B
1 mL	1280221	1155832	1.10762	2.92778	-0.00562024

The slope and intercept were obtained from the calibration curve generated by Analyst, and is presented in Appendix 1, page 32. The calibration points were weighted 1/x to provide better fit near the limit of detection

$$\text{AE B107137 found} = \frac{(1.10762 + (-0.00562024)}{2.92778} = 0.38 \text{ ng/mL}$$

Therefore sample 19DC-GS-10X-005 contains 0.38 ng/mL AE B107137 which agrees with the value reported in the raw data.

The % Recovery in each sample set was calculated in Microsoft Excel using the following formula:

$$\% \text{ Recovery} = \frac{(R-S)}{T} \times 100$$

Where: R = ng/mL of target analyte found in fortified sample
T = theoretical ng/mL in fortified sample
S = apparent ng/mL in control sample

0.38 ng/mL of AE B107137 was detected in water sample 19DC-GS-10X-005 fortified with 0.5 ng/mL AE B107137, and no residues were detected in the control samples.

Therefore:

$$\% \text{ AE B107137 Recovery} = \frac{(0.38-0)}{0.5} \times 100 = 76\%$$