Title

"Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil"

- Final Amended Report -

Test Substance

Diflufenican and its metabolites AE 0542291 and AE B107137

Data Requirement

US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation
OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007)

Completion Date

2019-09-11

Amended Report Completion Date

2020-07-20

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Sponsor

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Laboratory Study ID

037283

Sponsor Study ID

RADC0095

ABSTRACT

The purpose of this study was to validate the Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS" [1]. The validation was conducted in soil matrix to meet criteria for acceptance as defined by U.S. EPA and/or the European Commission for General Health and Consumer Protection.

The validation followed the US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method and OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation.

The validation also followed the OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007).

The method was successfully validated at LOQ (1.5 ppb) and 10x LOQ (15 ppb) in soil for diflufenican (AE F088657) and its metabolites AE 0542291 and AE B107137.

STUDY INFORMATION

STUDY TITLE

Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil

FRONTAGE LABORATORIES STUDY NUMBER

037283

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SCHEDULE OF EVENTS

Study Initiation Date:	March 26, 2019
Experimental Start Date:	April 15, 2019
Experimental Termination Date:	June 6, 2019
Study Completion Date:	Date report signed by Study Director

RETENTION OF DATA

Upon completion of the study, the complete study file including all original raw data will be submitted to the Frontage Laboratories Archives until transferred to Bayer CropScience.

CONDUCT OF THE STUDY

The study was conducted at the Frontage Laboratories Agrochemical Sciences Department Laboratories according to the Frontage Laboratories protocol, Document Number 037283-0, "Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil".

Personnel involved with the study were:

Penny Miner	Study Director
Kevin Walsh	Scientist
Emily Lenz	Associate Scientist I
Farhad Sayyarpour	Vice President

INTRODUCTION

This report describes the validation of Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS".

OBJECTIVE/PURPOSE

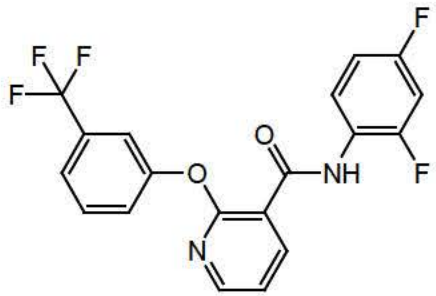
The purpose of this study is to validate Bayer Method "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS".

METHODS AND MATERIALS

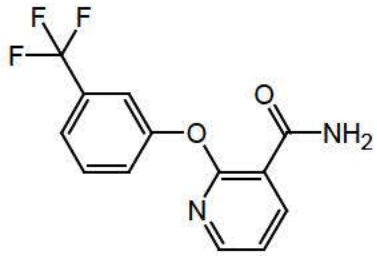
TEST SUBSTANCES

The sponsor supplied the test substances, Diflufenican, AE 0542291 and AE B107137. A copy of each certificate of analysis is located in Appendix A. The identity of the test substances was confirmed by LC-MS/MS using MRM (multiple reaction monitoring). The test substances were shipped on dry ice, received ambient, and stored frozen. Information concerning the test substances including purity is provided as follows:

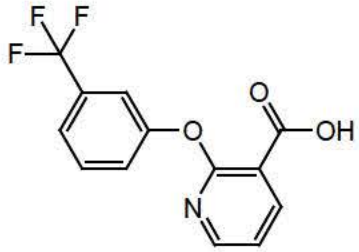
Name: Diflufenican (AE F088657)
CAS Number: 83164-33-44
Molecular Formula: $C_{19}H_{11}F_5N_2O_2$
Molecular Weight: 394.29 g/mol



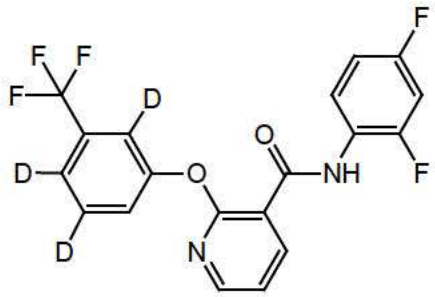
Name: AE 0542291
Molecular Formula: $C_{13}H_9F_3N_2O_2$
Molecular Weight: 282.22 g/mol



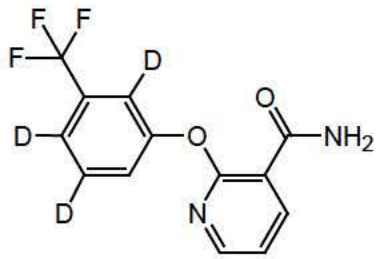
Name: AE B107137
Molecular Formula: $C_{13}H_8F_3NO_3$
Molecular Weight: 283.20 g/mol



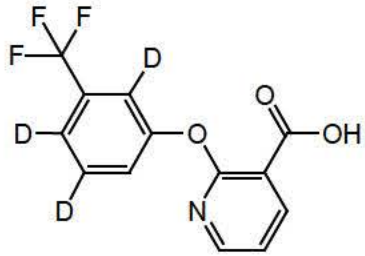
Name: Diflufenican-d3
Molecular Formula: $C_{19}H_8D_3F_5N_2O_2$
Molecular Weight: 397.32 g/mol



Name: AE 0542291-d3
Molecular Formula: $C_{13}H_6D_3F_3N_2O_2$
Molecular Weight: 285.24 g/mol



Name: AE B107137-d3
Molecular Formula: $C_{13}H_5D_3F_3NO_3$
Molecular Weight: 286.22 g/mol



TEST SYSTEM

The test system soil (Soil Sample MEDC0008IA-S001, Bayer Study Number MEDC0008, Iowa Terrestrial Field Dissipation) was provided by the Sponsor and was stored frozen before use.

SOLVENTS AND REAGENTS

- Formic acid, Fisher ACS Grade
- Acetone, Sigma HPLC Grade
- 2-propanol, Sigma HPLC Grade
- Ammonium Formate, Fisher Optima LC-MS Grade
- Acetonitrile (ACN), Sigma HPLC grade and Fisher Optima Grade
- Methanol (MeOH), Sigma HPLC grade and Fisher Optima Grade
- Acetic Acid, Fisher HPLC grade
- Water, Sigma and Fisher HPLC Grade
- 10 mM Ammonium Formate in 90:10 Water: Methanol with 120 µL per 1000 mL Formic Acid; prepared by adding 0.6306 g of Ammonium Formate to 100 mL of methanol, 900 mL of water, and 120µL of formic acid and mixing well.
- 10 mM Ammonium Formate in 10:90 Water: Methanol with 120 µL per 1000 mL Formic Acid; prepared by adding 0.6306 g of Ammonium Formate to 900 mL of methanol, 100 mL of water, and 120µL of formic acid and mixing well.
- Water: Methanol (90:10); prepared by adding 900 mL of water and 100 mL of methanol and mixing well.
- Acetonitrile: Water: Acetic Acid (400:100:3, v/v/v); prepared by adding 800 mL of acetonitrile, 200 mL of water and 6 mL of acetic acid and mixing well.

STOCK STANDARD SOLUTIONS, FORTIFICATION, AND CALIBRATION SOLUTIONS

Stock Solutions

Stock solutions of Diflufenican, AE 0542291 and AE B107137 were prepared at ~200 µg/mL in acetonitrile. Stock solutions of Diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3 were prepared at ~100 µg/mL in acetonitrile. The stock solutions were stored frozen when not in use.

Primary native standard solution preparation

The primary solutions were stored frozen when not in use.

Primary native standards	Standard Weight (mg)	Volume (mL)	Solvent	Final Concentration (µg/mL)
Diflufenican	5.13	25	ACN	204.79
AE 0542291	5.07	25	ACN	200.77
AE B107137	4.51	25	ACN	178.42

Primary Internal standards	Standard Weight (mg)	Volume (mL)	Solvent	Final Concentration (µg/mL)
Diflufenican-d_3	2.53	25	ACN	101.10
AE 0542291-d_3	2.26	25	ACN	90.40
AE B107137-d_3	2.34	25	ACN	93.60

Secondary mixed standard solution preparation

Intermediate calibration solution was prepared in acetonitrile as shown in the following table.

Primary native standard solutions	Primary standard concentration (µg/mL)	Aliquot from primary standards (mL)	Final volume (mL)	Final Concentration (µg/mL)
Diflufenican	204.79	1.221		
AE 0542291	200.77	1.245		
AE B107137	178.42	1.401	25	10.0
Diflufenican-d_3	101.10	2.473		
AE 0542291-d_3	90.40	2.765		
AE B107137-d_3	93.60	2.671	25	10.0

The intermediate solutions were stored frozen when not in use.

Calibration standard solutions were prepared from the intermediate solutions by dilution with water / methanol (9/1, v/v).

Additional mixed native secondary standard solution preparation

Diflufenican, AE 0542291 and AE B107137

Concentration of mixed native standard solution (µg/mL)	Aliquot Taken (mL)	Dilution Volume (mL)	Final Concentration (µg/mL)
10.0	2.5	25	1.0
1.0	2.5	25	0.1
0.1	2.5	25	0.01

Additional mixed internal standard solution preparation

Diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3

Concentration of mixed native standard solution (µg/mL)	Aliquot Taken (mL)	Dilution Volume (mL)	Final Concentration (µg/L) each analyte
10.0	2.5	25	1.0

Calibration standard solution preparation

Diflufenican, AE 0542291, AE B107137, Diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3

Calibration Standard Number	Concentration of Native Standard Solution used for dilution (µg/mL)	Concentration of Internal Standard Solution used for dilution (µg/mL)	Aliquot Native Taken (mL)	Aliquot Internal Standard Taken (mL)	Dilution Volume (mL)	Concentration of Native in Calibration Solution (ng/mL)	Concentration of IS in Calibration Solution (ng/mL)
Standard 7	1.0	1.0	0.50	0.05	10.0	50	5.0
Standard 6	1.0	1.0	0.20	0.05	10.0	20	5.0
Standard 5	0.1	1.0	0.50	0.05	10.0	5.0	5.0
Standard 4	0.1	1.0	0.20	0.05	10.0	2.0	5.0
Standard 3	0.01	1.0	0.50	0.05	10.0	0.50	5.0
Standard 2	0.01	1.0	0.20	0.05	10.0	0.20	5.0
Standard 1*	0.01	1.0	0.10	0.05	10.0	0.10	5.0

*Standard 1 is considered the LOD of the method.

ANALYTICAL METHODOLOGY

Fortification of matrix- Procedure

1. Weighed 25 grams ± 0.25 grams of matrix into a 4-oz. microwave vessel and added a magnetic stir bar.
2. Two control samples, 5 samples were spiked at 1.5 ppb and 5 samples were spiked at 15 ppb

Diflufenican, AE 0542291 and AE B107137

- 1.5 ppb (1.5 ng/g) fortified with 0.375 mL of 0.100 µg/mL native standard
- 15 ppb (15 ng/g) fortified with 0.375 mL of 1.00 µg/mL native standard

3. One tube was the reagent blank which followed the procedure below.
4. 50 mL of 400:100:3 (v/v/v) acetonitrile/water/acetic acid was added.
5. Samples placed into the microwave extractor.
6. Magnetic stirrer switched on.
7. Extracted for 15 minutes: From 0-3 minutes at 400 W (ambient temperature to approx. 70 °C) and held at 70 ºC from 3-15 minutes.
8. Added 0.250 mL of the 1.0-µg/mL mixed internal standard solution to each tube and mixed.
9. Transferred approximately 1-2-mL aliquot of supernatant to a micro-centrifuge tube and centrifuged for 5 minutes at >12000 g to remove fine particles.
10. Transferred a 1-mL aliquot to an LC vial.
11. The samples were analyzed for diflufenican, AE 0542291, AE B107137, diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3 using a LC-MS/MS analysis.
12. The sample set was completed in one 8-hour day.

LC-MS/MS Analysis

Separation of the analyte from the matrix was achieved by high performance liquid chromatography (HPLC). Quantitative LC-MS/MS analysis of the analyte in the samples utilized a highly specific and sensitive MRM (Multiple Reaction Monitoring) method. The analyte was identified by the coincidence of the retention time with that of the calibration standards, and quantified by integration of the peak area relative to the calibration curves.

The following are the LC-MS/MS system (controlled by the operating software, validated Analyst™ version 1.6.3) and the parameters used.

HPLC: Shimadzu
Shimadzu Pump LC-30AD
Shimadzu System Controller
Shimadzu Autosampler SIL-30ACMP
Shimadzu Column Oven CTO-20 AC

HPLC Conditions for Diflufenican, AE 0542291, AE B107137, Diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3

Column: Luna C18, 50 mm x 2.0 mm, 2.5 µm
Injection Volume: 10 µL
Column Temperature: 60 ºC
Solvent System:
Solvent A = 10 mM Ammonium Formate in 90:10 Water: Methanol
Solvent B = 10 mM Ammonium Formate in 10:90 Water: Methanol

Solvent Program:

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.1	0.5	100	0
0.5	0.5	100	0
3.00	0.5	0	100
3.50	0.5	0	100
3.51	0.5	100	0
5.01	Stop Run	100	0

Retention Time:

diflufenican and diflufenican-d_3 ~3.69 minutes
AE 0542291 and AE 0542291-d_3 ~ 2.97 minutes
AE B107137 and AE B107137-d_3 ~2.72 minutes

Mass Spectrometer: SCIEX API 6500

Mass Spectrometer Settings:

Positive ion mode
CUR: Curtain Gas 30
CAD: Collision Gas 8
GS1: Ion Source Gas 1 50
GS2: Ion Source Gas 2 50
TEM: Source Temp. 550 ºC
IS: Ion Transfer Voltage 5500

MRM:

Analyte ID	Polarity	Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (msec)	CE	CXP	DP
diflufenican	Positive	396	267.000	25	33	18	20
diflufenican C	Positive	396	247.000	25	49	14	20
diflufenican-d^3	Positive	399	267.894	25	33	18	20
diflufenican-d_3 C	Positive	399	248.000	25	49	14	20
AE 0542291	Positive	283.063	265.932	25	25	18	66
AE 0542291 C	Positive	283.063	245.995	25	25	22	66
AE 0542291-d_3	Positive	286.042	269.000	25	37	16	71
AE 0542291-d_3 C	Positive	286.042	247.930	25	37	16	71
AE B107137	Positive	284.050	246.078	25	37	20	76
AE B107137 C	Positive	284.050	265.889	25	27	16	76
AE B107137-d_3	Positive	287.029	247.937	25	37	16	61
AE B107137-d_3 C	Positive	287.029	267.000	25	27	16	76

METHODS OF CALCULATION

Recoveries

Calculation of Results

Analyst software was used to calculate the amount of diflufenican, AE 0542291, and AE B107137 in ng/mL for each sample and the percent recovery for the spiked samples. The standards were fit to the linear equation.

The general linear equation for a calibration curve is

y = mx +b, with 1/x weighting (x= calculated concentration -ng/mL, m=slope, b=intercept, y = native peak area)

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

$$x = \frac{(y-b)x\ D}{m}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (V1) x Final dilution volume (V3)}}{\text{Initial sample wt.(W)} \quad \text{Aliquot taken (V2)}}$$

Where: W = 25 g
V_1 = 50 mL
V_2 = 1.0 mL
V_3 = 1.0 mL

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

RESULTS AND DISCUSSION

The purpose of this study was to validate the Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS" [1].

The validation was conducted in soil to meet criteria for acceptance as defined by U.S. EPA and/or the European Commission for General Health and Consumer Protection.

The guideline is US EPA Test Guideline OCSPP 860.1340(c)(1)(2). The validation was considered successful if the mean recovery at each spiking level at or above the LOQ should be between 70% and 120% of the fortified concentration for analyte and the relative standard deviation (RSD, a.k.a. coefficient of variation, CV) of replicate measurements of recoveries did not exceed the target level of plus or minus 20%.

The first attempt of the method validation failed with LOQ's and 10X LOQ's outside of the acceptable 70% and 120% of the fortified concentration for each analyte and the relative standard deviation (RSD) of replicate measurements of recoveries exceeding 20%. After review of the data, it was determined that the LC/MS instrument (Sciex API-4000) used did not have the sensitivity required for this method and the LC/MS instrumentation was switched to the AB Sciex Triple Quad 6500. The second attempt of the method validation failed with LOQ's and 10X LOQ's outside of the acceptable 70% and 120% of the fortified concentration for each analyte. The relative standard deviations for this trial were within the ±20% The data and method were thoroughly reviewed for differences. The conclusion was that the microwave (model, conditions, etc.) was the only difference and was substantiated by adding the internal standard to the test system prior to microwaving the sample. The wattage was increased in order to maintain the microwave temperature of 70 ºC throughout the extraction duration.

The method was successfully validated for diflufenican and its metabolites AE 0542291 and AE B107137 in soil.

CONCLUSION

The Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS" was successfully validated for the determination of residues in soil samples.

DEVIATIONS

None

REFERENCES

1. Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS"
2. US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method
3. OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation
4. OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007) US EPA Test Guidelines OCSPP 860.1360: Multiresidue Method

PROTOCOL

Study Title:

Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil

Sponsor Study Number: RADC0095
Frontage Laboratories Study Number: 037283
Frontage Laboratories Document Number: 037283-0

Data Requirements:

US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method
OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation
OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007)

Testing Facility
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10845 Wellness Way
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Study Sponsor:
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TABLE OF CONTENTS

INTRODUCTION

This protocol describes the validation of Bayer Method DC-002-S18-01: "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS".

STUDY TITLE

Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil

FRONTAGE LABORATORIES STUDY NUMBER

037283

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OBJECTIVE

The United States Environmental Protection Agency (USEPA) published a data reporting guideline in the Federal Register on April 19, 1995 for Environmental Chemistry Methods (ECM). It included a requirement for registrants to validate methods at an independent laboratory prior to sending them to the Office of Pesticide Programs. The purpose of this study is to perform a validation of Bayer method DC-002-S18-01 "An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS".

This study will be conducted to satisfy guideline requirements described in US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method. OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007).
The validation will be performed by Frontage Laboratories in Concord, OH.

The study will be audited for compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160, by the Quality Assurance Unit of Frontage Laboratories.

SCHEDULE OF EVENTS

Proposed Experimental Start Date:	March 2019
Proposed Experimental Termination Date:	April 2019

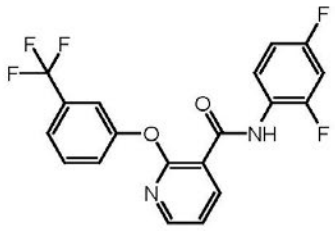
The actual experimental start and termination dates will be listed in the final report.

MATERIALS AND METHODS

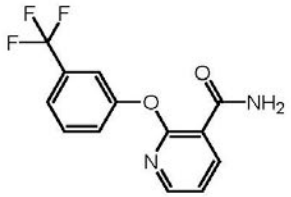
TEST SUBSTANCE

Test substance will be used to prepare analytical standard solutions and to fortify the test system to obtain percent recoveries. Test substance material will be provided by Bayer CropScience. The test substance will be used within the stability time-frame established by the supplier. Identity of the test substance will be verified by information on the label and/or certificate of analysis and confirmed by LC/MS analysis prior to the first sample trial. Information concerning the test substance is provided below.

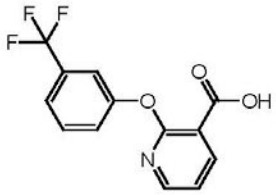
Name:	Diflufenican
CAS Number:	83164-33-44
Molecular Formula:	$C_{19}H_{11}F_5N_2O_2$
Molecular Weight:	394.29 g/mol



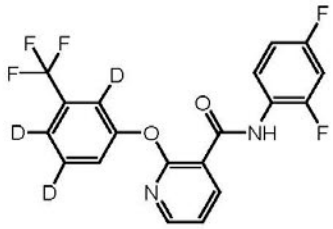
Name:	AE 0542291
Molecular Formula:	$C_{13}H_9F_3N_2O_2$
Molecular Weight:	282.22 g/mol



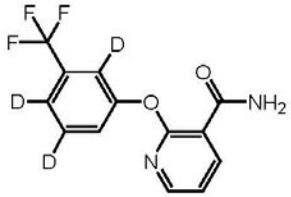
Name: AE B107137
Molecular Formula: $C_{13}H_8F_3NO_3$
Molecular Weight: 283.20 g/mol



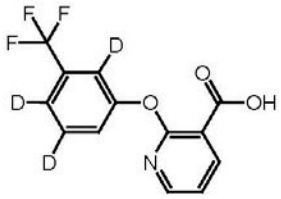
Name: Diflufenican-d3
Molecular Formula: $C_{19}H_8D_3F_5N_2O_2$
Molecular Weight: 397.32 g/mol



Name: AE 0542291-d3
Molecular Formula: $C_{13}H_6D_3F_3N_2O_2$
Molecular Weight: 285.24 g/mol



Name: AE B107137-d3
Molecular Formula: $C_{13}H_5D_3F_3NO_3$
Molecular Weight: 286.22 g/mol



CHARACTERIZATION AND RETENTION OF TEST SUBSTANCE

The Sponsor will assume the responsibility of retention of the sample of test substance, as specified in 40 CFR 160.195 and has the responsibility to characterize the test substance under GLP.

REAGENTS

All solvents will be HPLC grade or better in quality. Chemicals will be ACS reagent grade or better.

EXPERIMENTAL DESIGN

JUSTIFICATION OF THE TEST SYSTEM

Soil will be used for the validation. The use of soil is justified for validation of the method of analysis because a validated analytical method is required to detect and quantify diflufenican and its metabolites AE 0542291 and AE B107137 in soil.

SAMPLE HANDLING

All samples (provided by the Sponsor or by Frontage Laboratories) must be identified with a unique label or number assigned so that the study number, nature, test system, and date of collection are identified. Such identification should be either on the sample or cross-referenced to the sample and readily available on the paperwork.

ESTABLISHMENT OF THE METHOD

Prior to performing the validation, it will be necessary to establish the method, e.g., determine analyte retention times, instrument detection limits, linearity of instrument responses to a range of analyte concentrations. In general, Frontage Laboratories will demonstrate that the method is under control before initiating the validation.

Using the test substance, the testing facility will establish suitable LC/MS/MS parameters prior to initiation for the first sample trial. The testing facility will determine that it can obtain results similar to those shown in the method in terms of method sensitivity and

retention time of analytes. The elution profiles for the extracts will be determined and any needed adjustments made to ensure acceptable analyte recovery prior to initiation of the first sample set. The testing facility will additionally verify that the matrix control sample is either free of interferences or that interferences are negligible at the appropriate retention times (EPA guidelines require <30% of the LOQ concentration).

VALIDATION SETS

Bayer method DC-002-S18-01 will be validated in soil matrix for diflufenican and its metabolites AE 0542291 and AE B107137 using LC/MS/MS for both MRM transitions. A set of samples is defined as a reagent blank, two untreated control samples (UTCs), five UTCs fortified with diflufenican, AE 0542291 and AE B107137 in soil at the LOQ 1.5 ng/g, five UTCs fortified with diflufenican, AE 0542291 and AE B107137 at 10XLOQ as shown below.

Validation Set

1 reagent blank
2 UTCs
5 UTCs, each fortified at the LOQ
5 UTCs, each fortified at 10XLOQ

Total number of samples is 13 for each matrix.

All samples will be prepared at the same time using the same techniques in order to control bias.

METHOD PERFORMANCE

Frontage Laboratories will analyze a validation set by following instructions as detailed in the Bayer method DC-002-S18-01. Liquid chromatography and tandem mass spectrometry instrumentation and associated data system software specified in the method will be substituted for comparable equipment and software in the Frontage Laboratories laboratory. Quantitation will be conducted using Analyst™, and additional data reduction will be conducted using Microsoft Excel. The validation will be considered successful if the mean recovery at each spiking level at or above the LOQ is between 70% and 120% of the fortified concentration for analyte and the relative standard deviation (RSD, a.k.a. coefficient of variation, CV) of replicate measurements of recoveries does not exceed the target level of plus or minus 20%. The recoveries will be calculated with internal and external standard using peak areas from the same injection for both transitions.

PROPOSED DATA ANALYSIS AND STATISTICAL METHOD(S)

LC/MS data acquisition and calibration curve integration and regression analysis will be conducted using validated Analyst software TF 1.6.2.

Statistical methods used for this study will include Microsoft Excel™ (non-validated system) for measures of central tendency (i.e., percent of theoretical values, means, standard deviations, and relative standard deviations). All calculations performed by Microsoft Excel™ (non-validated system) will be peer verified for correctness. Intermediate calculation values presented in this report will be rounded only for discussion or presentation in tables, but the raw values themselves will be retained electronically until the final calculations are complete.

SAMPLE IDENTIFICATION

All sample data will be identified with at least the study number and unique code to signify matrix and replicate identification. This information will accompany the sample data in a manner that precludes error in the recording and storage of data. At a minimum, the study number will be used to label extracts and portions to be analyzed by LC/MS/MS.

DATA RECORDING AND RETENTION OF DATA

Records to be maintained will include, but not be limited, to the following:

- Description of experimental detail and methodology
- All relevant raw data
- Correspondence concerning the study
- Original signed protocol and any amendments
- Original signed report.

Upon completion of the study, the complete study file including all original raw data will be shipped to the sponsor (Bayer) via Frontage Laboratories archivist. A copy of the complete study file and all raw data will be retained at Frontage Laboratories.

DATA PRESENTATION

All operations, data and/or observations will be recorded on data collection forms or in a notebook and will be promptly signed or initialed and dated by the person conducting the operations, obtaining the data, and/or making the observations.

REPORT

A final report of the results of this study will be prepared by, or under the supervision of, the Study Director at the conclusion of the study. The report will include, but will not be limited to, the following:

- Name, address, and telephone number of study director and other contact person for the validation laboratory.
- Description of the analytical method.
- All recovery and control values for matrix that were obtained during all validation trials.
- Representative chromatograms/spectra for each analyte in each matrix.
- Description of the instruments used and operating parameters.
- Description of any problems encountered and a written description of any changes or modifications that were made during the validation.
- Correspondence with the method developer
- Any steps considered critical, i.e., steps where little variation is allowable or directions must be followed precisely.
- The number of worker-hours required to complete one set of samples.
- The number of calendar days required for one set of samples.
- A statement of adherence to FIFRA GLP standards under 40 CFR 160.12.

PROTOCOL AMENDMENTS AND DEVIATIONS

All agreed upon amendments will be expressed in writing, and signed and dated by the Study Director. Protocol amendments may also be signed by the Sponsor, or the Sponsor may provide their approval by means of a Sponsor Approval Form. Copies of the signed amendments and any Sponsor Approval Forms will be returned to the Study Director and appended to the protocol.

Deviations from the protocol, if any, will be documented and reported.

GLP COMPLIANCE

The described study will be conducted in accordance with the U.S. Environmental Protection Agency's "Good Laboratory Practice Standards" as published in 40 CFR Part 160. This study will be routinely examined by Frontage Laboratories Quality Assurance Unit personnel for compliance with GLPs, the protocol, and specific SOPs.

STATISTICAL METHODS AND BIAS CONTROL

When applicable, mean, standard deviation, and relative standard deviation will be used in the calculations. Bias will be controlled by preparing all samples at the same time, use of replicates and using the same techniques.

REFERENCES

1. US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method
2. US EPA Test Guideline OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation
3. OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007)