Title

"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 -

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

31-Oct-2019

Author

Penny Miner, M.S.

Testing Facility

Frontage Laboratories
10845 Wellness Way
Concord, OH 44077

Sponsor

Bayer CropScience
800 Lindbergh Blvd
St. Louis, MO 63167

Laboratory Study ID

037617

Sponsor Study ID

RADC0094

ABSTRACT

The purpose of this study was to validate the Bayer Method DC-004-W19-01: "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" [1]. The validation was conducted in pond water 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 (0.05 ppb) and 10x LOQ (0.5 ppb) in pond water for diflufenican (AE F088657) and its metabolites AE 0542291 and AE B107137.

STUDY INFORMATION

STUDY TITLE

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

FRONTAGE LABORATORIES STUDY NUMBER

037617

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TESTING FACILITY

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Agrochemical Sciences
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Concord, OH 44077

SCHEDULE OF EVENTS

Study Initiation Date:	September 4, 2019
Experimental Start Date:	September 19, 2019
Experimental Termination Date:	September 28, 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 037617-0, "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".

Personnel involved with the study were:

Penny Miner	Study Director
Joe DeLisio	Associate Scientist II
Emily Lenz	Associate Scientist I
Farhad Sayyarpour	Vice President

INTRODUCTION

This report describes the validation of Bayer Method DC-004-W19-01: "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".

OBJECTIVE/PURPOSE

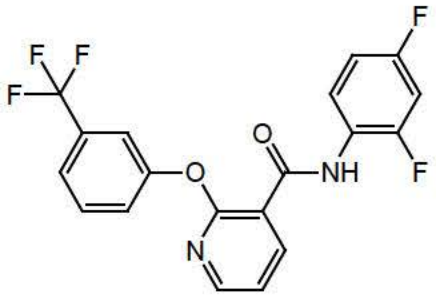
The purpose of this study is to validate Bayer Method "An Analytical Method for the Determination of Residues of AE F088657 and its metabolites AE 0542291 and AE B107137 in Water Using LC/MS/MS".

METHODS AND MATERIALS

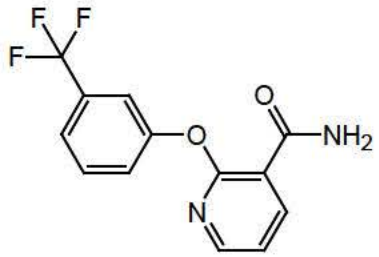
TEST SUBSTANCES

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 frozen, and stored frozen. Information concerning the test substances including purity is provided as follows:

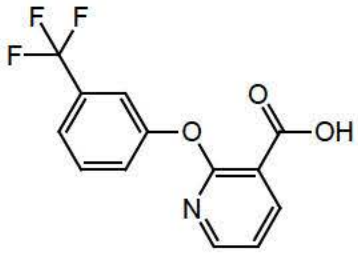
Name:	Diflufenican (AE F088657)
CAS Number:	83164-33-44
Molecular Formula:	$C_{19}H_{11}F_5N_2O_2$
Molecular Weight:	394.29 g/mol



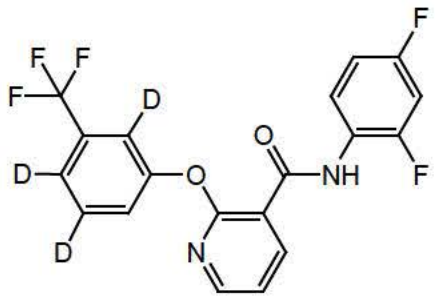
Name:	AE 0542291
Molecular Formula:	$C_{13}H_9F_3N_2O_2$
Molecular Weight:	282.22 g/mol



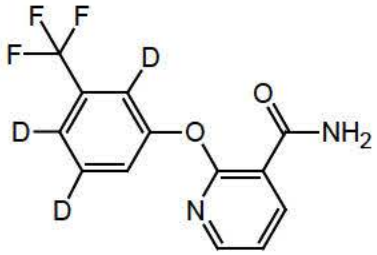
Name:	AE B107137
Molecular Formula:	$C_{13}H_8F_3NO_3$
Molecular Weight:	283.20 g/mol



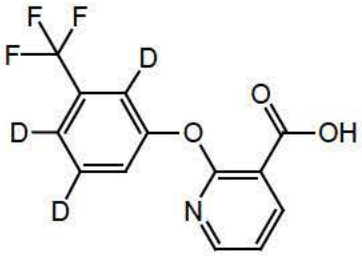
Name: Diflufenican-d3
Molecular Formula: $C_{19}H_8D_3F_5N_2O_2$
Molecular Weight: 397.32 g/mol



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, water, was provided by the Testing Facility and was stored refrigerated before use.

SOLVENTS AND REAGENTS

- Formic acid, Fisher ACS Grade
- Ammonium Formate, Fisher Certified ACS Grade
- Acetonitrile (ACN), Sigma HPLC grade and Fisher Optima Grade
- Methanol (MeOH), Fisher HPLC Grade
- Water, Fisher HPLC Grade
- 10 mM Ammonium Formate in 90:10 Water: Methanol with 120 µL per 1000 mL Formic Acid; prepared by adding 0.6305 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 0.6308 g of Ammonium Formate to 900 mL of methanol, 100 mL of water, and 120µL of formic acid and mixing well.

STOCK STANDARD SOLUTIONS, FORTIFICATION, AND CALIBRATION SOLUTIONS

Stock Solutions

Stock solution of Diflufenican was prepared at ~400 µg/mL in acetonitrile. Stock solutions of AE 0542291 and AE B107137 were prepared at ~560 µg/mL in acetonitrile. Stock solutions of Diflufenican-d_3, AE 0542291-d_3 and AE B107137-d_3 were prepared at ~200 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	4.06	10	ACN	405.19
AE 0542291	5.67	10	ACN	561.33
AE B107137	0.99	1.75	ACN	559.49

Primary Internal standards	Standard Weight (mg)	Volume (mL)	Solvent	Final Concentration (µg/mL)
Diflufenican-d_3	2.06	10	ACN	205.79
AE 0542291-d_3	2.08	10	ACN	208.00
AE B107137-d_3	2.10	10	ACN	210.00

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	405.19	0.617		
AE 0542291	561.33	0.445		
AE B107137	559.49	0.447	25	10.0
Diflufenican-d_3	205.79	0.061		
AE 0542291-d_3	208.00	0.060		
AE B107137-d_3	210.00	0.060	25	0.50

The intermediate solutions were stored frozen when not in use.

Calibration standard solutions were prepared from the intermediate solutions by dilution with acetonitrile.

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
0.01	1.0	25	0.0004

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
0.5	0.2	25	0.0004

Calibration standard solution preparation

Calibration solutions was prepared in acetonitrile: water (20:80, v:v) as shown in the following table.

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	0.5	0.050	0.025	25	2	0.5
Standard 6	1.0	0.5	0.025	0.025	25	1	0.5
Standard 5	0.1	0.5	0.125	0.025	25	0.5	0.5
Standard 4	0.1	0.5	0.063	0.025	25	0.25	0.5
Standard 3	0.1	0.5	0.025	0.025	25	0.10	0.5
Standard 2	0.01	0.5	0.125	0.025	25	0.05	0.5
Standard 1*	0.01	0.5	0.050	0.025	25	0.02	0.5

*Standard 1 is considered the LOD of the method.

ANALYTICAL METHODOLOGY

Fortification of matrix- Procedure

1. Removed 10 - 20 mL of water from the jar and placed into a scintillation vial.
2. Transferred 1 mL of water for each sample into a LC/MS vial using a pipet.
3. Two control samples, 5 samples were spiked at 0.05 ppb and 5 samples were spiked at 0.5 ppb

Diflufenican, AE 0542291 and AE B107137

- 0.05 ppb (0.05 ng/g) fortified with 0.125 mL of 0.0004 µg/mL native standard
- 0.5 ppb (0.5 ng/g) fortified with 0.05 mL of 0.010 µg/mL native standard

4. One vial was the reagent blank which followed the procedure below.
5. Added 0.125 mL of 0.004 µg/mL mixed internal standard to the water.
6. Capped the LC/MS vial and mixed well.
7. 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.
8. 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: Phenomenex C(18) HST, 50 mm x 2.0 mm, 2.5 µm
Injection Volume: 90 µL
Column Temperature: 40 ºC
Solvent System:

Solvent A = 10 mM Ammonium Formate in 90:10 Water: Methanol with 120 µL of Formic Acid
Solvent B = 10 mM Ammonium Formate in 10:90 Water: Methanol with 120 µL of Formic Acid

Solvent Program:

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.5	1.0	100	0
3.00	1.0	0	100
3.50	1.0	0	100
3.51	1.0	100	0
4.01	Stop Run	100	0

Retention Time:

diflufenican and diflufenican-d_3 ~3.20 minutes
AE 0542291 and AE 0542291-d_3 ~ 2.51 minutes
AE B107137 and AE B107137-d_3 ~2.28 minutes

Mass Spectrometer: SCIEX API 6500

Mass Spectrometer Settings:

Positive ion mode
CUR: Curtain Gas 50
CAD: Collision Gas 9
GS1: Ion Source Gas 1 70
GS2: Ion Source Gas 2 80
TEM: Source Temp. 600 ºC
IS: Ion Transfer Voltage 5500

MRM:

Analyte ID	Polarity	Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (msec)	CE	CXP	DP
diflufenican	Positive	395.2	266.000	35	33	22	171
diflufenican C	Positive	395.2	246.000	35	51	14	171
diflufenican-d^3	Positive	398.1	268.000	35	35	16	121
diflufenican-d_3 C	Positive	398.1	248.000	35	49	14	121
AE 0542291	Positive	283.1	266.000	35	29	18	51
AE 0542291 C	Positive	283.1	246.000	35	39	14	51
AE 0542291-d_3	Positive	286.2	268.000	35	27	16	76
AE 0542291-d_3 C	Positive	286.2	220.000	35	49	12	76
AE B107137	Positive	284.1	266.000	35	27	14	91
AE B107137 C	Positive	284.1	238.000	35	39	20	91
AE B107137-d_3	Positive	287.1	268.000	35	27	14	71
AE B107137-d_3 C	Positive	287.1	240.000	35	41	14	130

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/mL was determined using the following equation:

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

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.

REFERENCES

1. Bayer Method DC-004-W19-01: "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"
2. US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method
3. US EPA Test Guideline 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)

PROTOCOL

Study Title:

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

Sponsor Study Number: RADC0094
Frontage Laboratories Study Number: 037617
Frontage Laboratories Document Number: 037617-0

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)

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Agrochemical Services
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10845 Wellness Way
Concord, OH 44077

Study Sponsor:
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St. Louis, MO 63167

TABLE OF CONTENTS

INTRODUCTION

This protocol describes the validation of Bayer Method DC-004-W19-01: "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".

STUDY TITLE
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

FRONTAGE LABORATORIES STUDY NUMBER
037617

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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-004-W19-01: "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".

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, LLC 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 Concord.

SCHEDULE OF EVENTS

Proposed Experimental Start Date:	September 2019
Proposed Experimental Termination Date:	September 2019

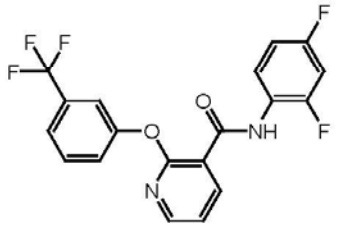
The actual experimental start and termination dates will be listed in the final report.

MATERIALS AND METHODS

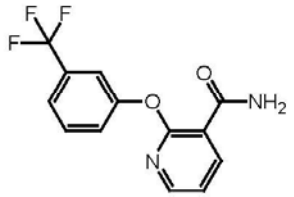
TEST SUBSTANCES

Test substances will be used to prepare analytical standard solutions and to fortify the test system to obtain percent recoveries. Test and reference substances will be provided by Bayer CropScience. The test substances will be used within the stability time-frame established by the supplier. Identity of the test substances 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 substances is provided below.

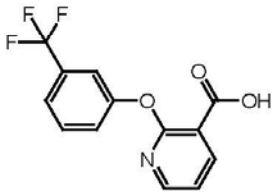
Name:	AE F088657
Alternative Name:	Diflufenican
CAS Number:	83164-33-44
Molecular Formula:	$C_{19}H_{11}F_5N_2O_2$
Molecular Weight:	394.29 g/mol



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

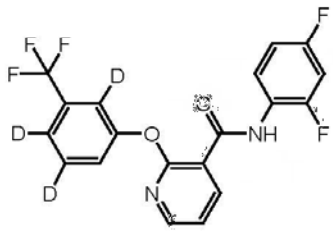


Name: AE B107137
Molecular Formula: $C_{13}H_8F_3NO_3$
Molecular Weight: 283.20 g/mol

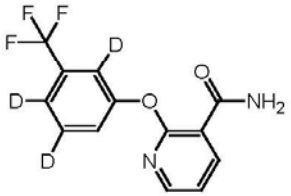


REFERENCE SUBSTANCES

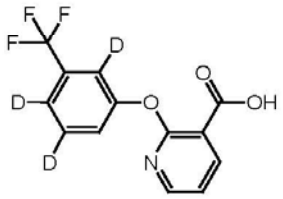
Name: AE F088657-d3
Molecular Formula: $C_{19}H_8D_3F_5N_2O_2$
Molecular Weight: 397.32 g/mol



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

If the study duration is longer than 4 weeks, the Sponsor will assume the responsibility of retention of the sample of test and reference substances, as specified in 40 CFR 160.195. The Sponsor has the responsibility of stability, solubility and characterization of the test substances 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

Water will be used for the validation. The use of water is justified for validation of the method of analysis because a validated analytical method is required to detect and quantify AE F088657 and its metabolites, AE 0542291 and AE B107137 in water.

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-004-W19-01 will be validated in water matrix for AE F088657 (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 AE F088657 and its metabolites AE 0542291 and AE B107137 at the LOQ 0.05 ng/mL, five UTCs fortified with AE F088657 and its metabolites, AE 0542291 and AE B107137 at 10x LOQ as shown below.

Validation Set

1 reagent blank
2 UTCs
5 UTCs, each fortified at the LOQ with diflufenican, AE 0542291 and AE B107137
5 UTCs, each fortified at 10XLOQ with diflufenican, AE 0542291 and AE B107137

Total number of samples is 13 for each fortification set.

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 no. DC-004-W19-01. 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 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 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 1.6.3.

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 retain 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)