1. Introduction

1.1 Background

Carfentrazone-ethyl (F8426, CAS#: 128639-02-1) is an herbicide in the aryl triazolinone family produced by FMC Corporation. Carfentrazone-ethyl has four common metabolites in soil: F8426-benzoic acid (F8426-BAc), F8426-propionic acid (F8426-PAc), F8426-chloropropionic acid (F8426-Cl-PAc), and F8426-cinnamic acid (F8426-CAc). Primera Analytical Solutions Corp. (hereafter referred to as PASC) has been contracted by FMC Corporation to conduct an independent laboratory validation (ILV) to demonstrate that the FMC analytical methods reported in RAN-0270M can be performed with acceptable recoveries for quantitative determination of carfentrazone-ethyl and its four metabolites in soil by GC-MS. This study was conducted as part of a data call-in for the EPA registration review. The study protocol is provided in Attachment III.

1.2 Purpose

This report summarizes the validation of a quantitative GC-MS method for determination of carfentrazone-ethyl and its four metabolites in soil. The results demonstrate that the analytical method is suitable for its intended use.

1.3 Scope

This report applies to the validation method for the analysis of carfentrazone-ethyl and its four metabolites in soil, which was previously developed by FMC and reported in RAN-0270M, according to validation protocol PASC-PRT-0557.

2. References

2.1 Report RAN-0270M

2.2 PASC-SOP-0402, Version No. 09: "Deviation Resolution"

2.3 EPA OCSPP 850.6100, "Ecological Effects Test Guidelines-Data Reporting for Environmental Chemistry Methods", EPA 712-C-001, January 2012

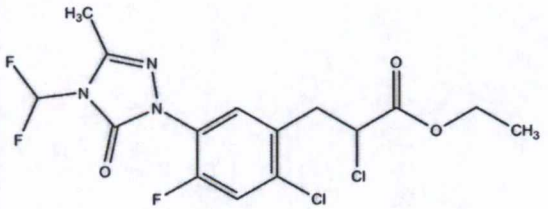
2.4 EPA OPP Pesticide Registration (PR) Notice 2011-3, Standard Format for Data Submitted Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Certain Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), January 2012.

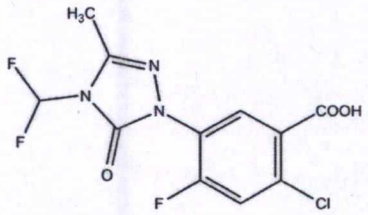
3. Materials and Equipment

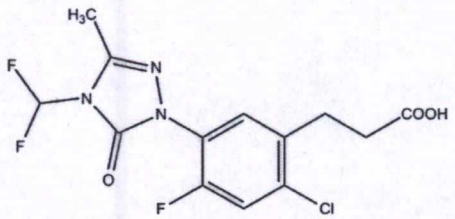
3.1 Chemicals and Reagents

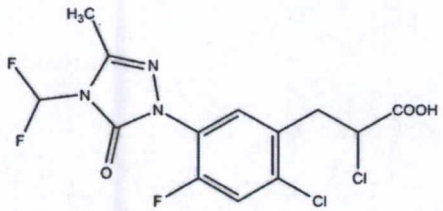
Analytes

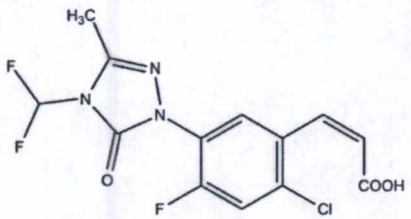
The reference standards for Carfentrazone-ethyl and its four metabolites were provided by FMC.

Common Name:	F8426 (carfentrazone-ethyl)
Chemical Name (IUPAC):	ethyl 2-chloro-3-(2-chloro-5-(4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-4-fluorophenyl)propanoate
CAS Registry No.:	128639-02-1
Chemical Structure:	
Molecular Formula:	$C_{15}H_{14}Cl_2F_3N_3O_3$
Molecular Mass:	412.191
Supplier	FMC Agricultural Products
FMC Number	116426
Purity	95.5%
Expiration Date	February 2017

Common Name:	F8426-benzoic acid (F8436-BAc)
Chemical Name (IUPAC):	2-chloro-5-(4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-4-fluorobenzoic acid
CAS Registry No.:	N/A
Chemical Structure:	
Molecular Formula:	$C_{11}H_7ClF_3N_3O_3$
Molecular Mass:	321.640
Supplier	FMC Agricultural Products
FMC Number	97083
Purity	99.4%
Expiration Date	March 2015

Common Name:	F8426-proprionic acid (F8426-PAc)
Chemical Name (IUPAC):	3-(2-chloro-5-(4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-4-fluorophenyl)propanoic acid
CAS Registry No.:	N/A
Chemical Structure:	
Molecular Formula:	$C_{13}H_{11}ClF_3N_3O_3$
Molecular Mass:	349.693
Supplier	FMC Agricultural Products
FMC Number	125165
Purity	98.8%
Expiration Date	March 2015

Common Name:	F8426-chloropropionic acid (F8426-Cl-PAc)
Chemical Name (IUPAC):	2-chloro-3-(2-chloro-5-(4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-4-fluorophenyl)propanoic acid
CAS Registry No.:	N/A
Chemical Structure:	
Molecular Formula:	$C_{13}H_{10}Cl_2F_3N_3O_3$
Molecular Mass:	383.005
Supplier	FMC Agricultural Products
FMC Number	124161
Purity	98.0%
Expiration Date	March 2017

Common Name:	F8426-cinnamic acid (F8426-CAc)
Chemical Name (IUPAC):	(Z)-3-(2-chloro-5-(4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-4-flurophenyl)acrylic acid
CAS Registry No.:	128639-10-1
Chemical Structure:	
Molecular Formula:	$C_{13}H_9ClF_3N_3O_3$
Molecular Mass:	347.677
Supplier	FMC Agricultural Products
FMC Number	125151
Purity	98.3%
Expiration Date	March 2015

Sample Matrix

Soil (PASC ID 130736, FMC ID UTC-SC-1-CTR-6) was provided by FMC Corporation. The soil was collected from Sycamore, Georgia, and has been classified as loamy sand. The GLP soil characterization data is presented in Attachment II.

Chemicals

Chemical	Supplier	Catalog Number
Acetonitrile	PHARMCO-AAPER	30000HPLC
Carbitol™	Sigma	597548
Diazald®	Aldrich	D28000
Ether, Anhydrous	J.T. Baker	9244-22
Ethyl Ether	PHARMCO-AAPER	373000000
Ethyl Acetate	PHARMCO-AAPER	3300000EP
n-Hexane	PHARMCO-AAPER	359095DIS
Methylene Chloride	PHARMCO-AAPER	31300HPLC
Milli-Q Water	Millipore Milli-Q Gradient A10	---
Potassium Hydroxide	Sigma-Aldrich	P5958
Sodium Chloride	J.T. Baker	3627-01
Sodium Sulfate, Anhydrous	Acros Organics	196640025
Sulfuric Acid	Sigma-Aldrich	320501

Reagent Solutions

Note: Solutions may be prepared in different volumes, as long as the components and proportions are not altered.

Reagent	Preparation
Reflux Solvent	800 mL ACN 200 mL Water
Hexane:Ethyl Acetate (95:5, v/v)	114 mL n-Hexane 6 mL Ethyl Acetate
Hexane:Ethyl Acetate (8:2, v/v)	224 mL n-Hexane 56 mL Ethyl Acetate
10% H_2SO_4	5.1 mL Sulfuric Acid 44.9 mL Milli-Q water
Acetonitrile:Water (8:2, v/v)	1600 mL Acetonitrile 400 mL Milli-Q water

3.2 Equipment

Equipment	Supplier/Model	Catalog Number
Analytical Balance	Mettler Toledo XS105 Mettler PE 3600	---
Diazomethane Generator Kit	Sigma Diazald® Kit with Clear-seal® Joints	Z100250
Roto-evaporator	Büchi Rotavapor R-114	---
Filter Paper	Whatman #4	1004 090
SPE Manifold	IST VacMaster	---
Glass Microsyringes	Various	---
Laboratory Glassware (beakers, flasks, reflux columns, Büchner funnels)	Various	---
SPE Columns (SI silica, 1 g/6 mL)	Agilent Bond Elut SI Silica, 1 g/6 mL	12256008
Gas Chromatograph	Agilent 6890N	---
Mass Spectrometer	Agilent 5973	---

4. Instrument Conditions/Parameters

4.1 Chromatographic Conditions

Column:	Agilent HP-5 25 m x 0.32 mm id, 0.52 µm film Part No. 19091J-112E
Carrier Gas:	Helium
Column Flow Rate:	1.5 mL/min
Inlet	
Inlet Mode:	Split
Split Ratio:	5:1
Split Flow:	7.5 mL/min
Total Flow:	11.9 mL/min
Injection Volume:	1 µL

Oven Temperature Program

Initial Temperature: 150°C **Initial Time:** 1.0 min

Rate	Final Temperature	Final Time
25°C/min	250°C	0.0 min
5°C/min	260°C	4.0 min
30°C/min	295°C	2.75 min

Total Run Time: 14.92 min

4.2 Mass Spectrometer Method Properties

General MS Acquisition Parameters for All Analytes	
GC-MS Interface Temperature:	280°C
MS Source Temperature:	230°C
MS Quad Temperature:	150°C
Solvent Delay:	5.0 minutes
Gain Factor:	5.00
Resulting EM Voltage:	1906
Scanning Mode:	SIM

4.3 Mass Transitions and Voltages

Analyte Group	Start Time (min)[1]	Resolution	Ion (*m/z*)	Dwell
F8426	7.10	High	330	100
F8426-BAc	5.00	High	335	100
F8426-PAc	6.20	High	303	100
F8426-Cl-PAc	6.75	High	326	100
F8426-CAc[2]	6.90	High	361	100

[1]The start time for each analyte also indicated the end time for the monitoring of the previous ion.
[2]F8426-CAc was initially monitored using ion *m/z* 326, but an interfering peak was observed at the same retention time. F8426-CAc was re-analyzed using ion *m/z* 361 to monitor instead.

5. Validation Procedures

The method validation was conducted from November 26, 2014 to December 30, 2014, at PASC in Princeton, New Jersey. The single standard stock solutions and the mixed fortification standard solution were prepared on November 26, 2014. Soil samples were spiked with analytes and extracted on December 20, 2014. Aliquots of the sample extracts were derivatized and brought through the clean-up and final dilution procedures on December 21, 2014. An aliquot of each of the two control sample extracts was combined, fortified, and brought through the derivatization, clean-up, and final dilution procedures to prepare the matrix-matched standard curve on December 21, 2014.

The analytical set was first run on December 22, 2014. The acceptance criteria for recovery and % RSD were met for the analytes F8426, F8426-PAc, and F8426-Cl-PAc. However, an interfering peak was observed in the control sample at the retention time of F8426-CAc when monitored at *m/z* 326. This interfering peak artificially increased the analyte recovery to above the acceptable levels. Additionally, the percent recovery of the 10×LOQ samples for F8426-BAc was slightly higher than the acceptable levels in the first analytical run, so the samples at the 10×LOQ fortification level were re-diluted from the final sample extracts.

The sequence was injected a second time on December 24, 2014, using the re-diluted 10×LOQ samples and monitoring F8426-CAc at *m/z* 361 instead of *m/z* 326, as recommended in the original method report RAN-0270M. However, the sequence stopped near the end, so the data was voided due to the lack of a bracketing standard at the end of the sequence.

The sequence was injected a third time on December 30, 2014, under the same conditions as the sequence run performed on December 24, 2014. Only the data for F8426-BAc and F8426-CAc was processed from this analytical sequence. No interfering peaks were observed under the new monitoring conditions, and the criteria for recovery and % RSD were met for these two analytes.

5.1 Diazomethane/Ether Reagent Preparation

A diazomethane solution was prepared in ether to use for the derivatization of the analytes.

Potassium hydroxide (3.6 g), Carbitol™ (21 mL), and ether (6 mL) were added to a reaction flask resting in a water bath set at 60-65°C. Diazald® (12.84 g) and ether (75 mL) were added to a separatory funnel connected to the reaction flask. The reaction flask was also connected to a condenser, which led to a collection flask resting in an ice bath. The Diazald® mixture was slowly introduced to the reaction flask, and the resultant vapor (the diazomethane/ether solution) was condensed and collected into the flask resting in the ice bath.

The diazomethane/ether solution was stored in a screw-capped vial in a freezer (-70°C) until use.

5.2 Stock and Fortification Standard Solution Preparation

Stock Standard Solution Preparation

Individual stock standard solutions were prepared for carfentrazone ethyl and each of the four metabolites. Each standard solution was prepared to a concentration of about 1 mg/mL in Class A volumetric flasks, using acetonitrile as the diluent. Weights were not corrected for purity, and the stock standard solutions were stored refrigerated after preparation. The preparation of the stock standard solutions is summarized in Table 1.

Table 1. Preparation of the Stock Standard Solutions

Analyte	Purity	Weight (mg)	Final Volume (mL)	Final Concentration (mg/mL)
Carfentrazone-ethyl	95.5%	25.41	25	1.0164
F8426-BAc	99.4%	25.38	25	1.0152
F8426-PAc	98.8%	25.44	25	1.0176
F8426-Cl-PAc	98.0%	25.02	25	1.0008
F8426-CAc	98.3%	24.00	25	0.9600

Fortification Standard Solution Preparation

A single mixed fortification solution was prepared containing carfentrazone-ethyl and each of the four metabolites. The standard was prepared to a concentration of about 10 µg/mL for each analyte, using acetonitrile as the diluent. The preparation of the fortification solution is summarized in Table 2.

Table 2. Preparation of the Fortification Solution

Analyte in Stock Solution	Concentration in Stock Solution (mg/mL)	Volume (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Carfentrazone-ethyl	1.0164	0.0994	10	10
F8426-BAc	1.0152	0.0985		
F8426-PAc	1.0176	0.0983		
F8426-Cl-PAc	1.0008	0.100		
F8426-CAc	0.9600	0.104		

5.3 Residue Sample Preparation

Fortification

To each of twelve 250 mL boiling flasks, 40.0 g of soil were transferred and fortified with the mixed fortification solution as outlined in Table 3.

Table 3. Soil Sample Fortification

Sample Type	Number of Replicates	Soil Weight (g)	Fortification Solution Concentration (µg/mL)	Fortification Volume (mL)	Fortification Level (ppb)
Reagent Blank	1	0	N/A	0	0
Control	2	40.0	N/A	0	0
LOQ	5	40.0	10	0.020	5
10×LOQ	5	40.0	10	0.20	50

Sample Reflux and Extraction

To each fortified sample, control sample, and reagent blank, 100 mL of reflux solvent (acetonitrile:water, 8:2 v/v) was added. The flasks were gently swirled and were placed on a hot plate under a cold water condenser. The samples were refluxed for one hour, with occasional manual swirling, and then were allowed to cool.

Each sample was vacuum filtered through a Büchner funnel with a Whatman #4 filter paper. The boiling flask was rinsed with about 25 mL of reflux solvent, and all of the filtrate was collected into a clean 250 mL flask.

The filtrate was evaporated using a roto-evaporator in a water bath set at 55°C to a final volume of about 15 mL. After evaporation, the volume was adjusted to about 25 mL with Milli-Q water. The samples were then acidified with 0.5 mL of 10% H_2SO_4 and were transferred to a 250 mL separatory funnel.

The samples were extracted with 37.5 mL of methylene chloride and approximately 1 teaspoon of sodium chloride. The organic phase was collected and was passed through a bed of granular anhydrous sodium sulfate in a glass funnel. The sample was extracted in the same way a second time with another 37.5 mL aliquot of methylene chloride. The organic phase extracts were combined and transferred to a 100 mL graduated cylinder, and the final volume of the extract solution was adjusted to 80 mL with methylene chloride.

Sample extracts were stored refrigerated until derivatization.

Sample Derivatization

A 40 mL aliquot of the sample extract was transferred to a 250 mL beaker. The aliquot was evaporated just to dryness on a warm steam table (about 60-70°C) under a gentle stream of nitrogen. Ethyl ether (5 mL) and diazomethane/ether solution (1 mL) were added to the dried sample. The beaker was gently swirled and the sample was allowed to derivatize undisturbed for 30 minutes.

After 30 minutes, 10 mL of hexane was added and each sample was gently swirled to mix. The sample was concentrated on a warm steam table (about 60-70°C) under a gentle stream of nitrogen to a volume of 1-2 mL. Hexane (10 mL) was added to each concentrated sample.

Silica SPE Clean-up

Each SPE cartridge was conditioned with 10 mL of hexane. The derivatized sample was passed through the cartridge, followed by 5 mL of hexane:ethyl acetate (95:5, v/v). The sample was then eluted with 10 mL of hexane:ethyl acetate (8:2, v/v) and was collected into a 13 mL graduated centrifuge tube.

Final Concentration

The samples were concentrated under a gentle stream of nitrogen to less than 0.5 mL, then were brought to a final volume of 0.5 mL with ethyl acetate.

Dilution of 10×LOQ Samples

The 10×LOQ samples were diluted to 20% of their original concentrations using ethyl acetate as the diluent.

5.4 Matrix-Matched Standard Curve Preparation

Stock Derivatized Standard Preparation

A 20 mL aliquot was taken from each of the two control sample extracts. The aliquots were combined, for a total volume of 40 mL, and were transferred to a 250 mL beaker. The combined aliquot was evaporated just to dryness on a warm steam table (about 60-70°C) under a gentle stream of nitrogen.

The dried extracted was then fortified with 160 µL of the fortification standard solution that was prepared in Section 5.3.

Derivatization of this standard was then continued with the addition of ethyl ether (5 mL) and diazomethane/ether (1 mL). The stock solution was carried through the rest of the sample preparation steps up to the Final Concentration. For the Final Concentration step, the stock derivatized standard was concentrated under a gentle stream of nitrogen to less than 0.5 mL, then was brought to a final volume of 2 mL with ethyl acetate. The concentration of the stock derivatized standard was 800 ng/mL.

Serial Dilution

The standard curve was prepared by serially diluting the stock derivatized standard solution with ethyl acetate, using a glass micro-syringe, according to Table 4.

Table 4. Preparation of the Standard Curve

Concentration of Take Solution (ng/mL)	Volume of Take Solution (µL)	Volume of Ethyl Acetate (µL)	Final Concentration (ng/mL)
800	200	200	400
400	200	200	200
200	200	200	100

5.5 Modifications to the Original Method

The following modifications were applied to the methods:

5.6 Injection Sequence

Five replicate samples at two fortification levels were used to evaluate the method efficiency. Calibration standards were injected within the analysis set to ensure detector linearity and stable response.

The validation set contained at least one reagent blank, two unfortified matrix controls, five matrix control samples fortified at LOQ (5.00 ppb) and five matrix control samples fortified at 10×LOQ (50.0 ppb). The injection sequences for the two valid sample runs are outlined in Tables 5 and 6.

Table 5. Injection Sequence (for F8426, F8426-Cl-PAc, and F8426-PAc)

Injection Sequence	Sample Type
1	Diluent
2	Standard (100 ng/µL)
3	Standard (200 ng/µL)
4	Standard (400 ng/µL)
5	Standard (800 ng/µL)
6	Diluent
7	Reagent Blank
8	Control-1
9	Control-2
10	Standard (100 ng/µL)
11	LOQ-1
12	LOQ-1
13	LOQ-3
14	LOQ-4
15	LOQ-5
16	Standard (200 ng/µL)
17	10×LOQ-1
18	10×LOQ-2
19	10×LOQ-3
20	10×LOQ-4
21	10×LOQ-5
22	Standard (400 ng/µL)
23	Standard (800 ng/µL)

Table 6. Injection Sequence (for F8426-CAc and F8426-BAc)

Injection Sequence	Sample Type
1	Standard (100 ng/µL)
2	Standard (200 ng/µL)
3	Standard (400 ng/µL)
4	Standard (800 ng/µL)
5	Diluent
6	Reagent Blank
7	Control-1
8	Control-2
9	Standard (100 ng/µL)
10	LOQ-1
11	LOQ-1
12	LOQ-3
13	LOQ-4
14	LOQ-5
15	Standard (200 ng/µL)
16	10×LOQ-1
17	10×LOQ-2
18	10×LOQ-3
19	10×LOQ-4
20	10×LOQ-5
21	Standard (400 ng/µL)
22	Standard (800 ng/µL)