

FMC 842ILV12R1		PASC 058-0754
TITLE: Independent Laboratory Validation of the Method for the Analysis of Carfentrazone-Ethyl and Its Metabolites in Soil by LC-MS/MS		
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1.0 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 major metabolites in soil: F8426-chloropropionic acid (F8426-Cl-PAc), F8426-cinnamic acid (F8426-CAc), F8426-propionic acid (F8426-PAc), and F8426-benzoic acid (F8426-BAc). 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 LC-MS/MS. This study was conducted as part of a data call-in for the EPA registration review.

1.2 Purpose

This report summarizes the validation of a quantitative LC/MS/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 were previously developed by FMC and reported in RAN-0270M, according to validation protocol PASC-PRT-0274.

2.0 References

- 2.1 Study Protocol PASC-PRT-0274, "Independent Laboratory Validation of the Method for the Analysis of Carfentrazone-Ethyl and Its Metabolites in Soil by LC-MS/MS"
- 2.2 FMC Corp. Report RAN-0270M, FMC Study# 842E4194E1
- 2.3 PASC-SOP-0512 V01, "GLP Deviation Resolution"

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- 2.4 EPA OCSP 850.6100, "Ecological Effects Test Guidelines-Data Reporting for Environmental Chemistry Methods", EPA 712-C-001, January 2012
- 2.5 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.

3.0 Materials and Equipment

3.1 Materials and Chemicals

3.1.1 Analytes

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

1) Carfentrazone-ethyl

Common name: F8426
 Chemical Name: Ethyl, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1 H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate
 CAS No.: 128639-02-1
 Molecular formula: C₁₅H₁₄Cl₂F₃N₃O₃
 Molecular weight: 412.19
 Supplier: FMC
 Purity: 95.5%
 Expiration Date: Feb-2017
 FMC ID: 116426

- 2) F8426-chloropropionic acid (F8426-Cl-PAC), metabolite, 98.0%, FMC-124161, Expiration date: Mar-2017
- 3) F8426-cinnamic acid (F8426-CAC), metabolite, 98.3%, FMC-125151, Expiration date: Mar-2015
- 4) F8426-propionic acid (F8426-PAC), metabolite, 98.8%, FMC-125165, Expiration date: Mar-2015

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- 5) F8426-benzoic acid (F8426-BAc), metabolite, 99.4%, FMC-97083, Expiration date: Mar-2015

3.1.2 Matrix

Soil (PASC ID 120022) was provided by FMC Corporation. The non-GLP soil characterization data can be found in the Attachment II.

3.1.3 Reagents and Materials

- 1) Formic Acid (96%), ACS grade, Spectrum
- 2) Acetonitrile, HPLC grade, Pharmco-Aaper
- 3) Ammonium Bicarbonate (NH₄HCO₃) reagent grade, J.T.Baker
- 4) Sulfuric Acid, ACS grade, Sigma-Aldrich
- 5) Hydrogen Chloride, ACS grade, Spectrum
- 6) Sodium Chloride, ACS grade, BDH
- 7) Sodium Sulfate (Anhydrous), ACS grade, EMD
- 8) Methylene Chloride, HPLC grade, Burdick & Jackson
- 9) Whatman filter papers, GF/A 12.5 cm glass microfilters

3.2 Equipment

- 3.2.1 SHIMADZU SIL-HTC Autosampler and SHIMADZU LC-10ADvp pumps coupled with Applied Biosystems 5000 Triple Quadrupole mass spectrometer (LETS #154)
- 3.2.2 Analytical Balance capable of weighing to 0.1 mg (LETS #169), daily verification
- 3.2.3 AB SciEx Analyst© 1.4.2 Software
- 3.2.4 Centrifuge, Model SorVall T6000, Thermal Scientific (LETS# 76)

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4.0 Instrument Conditions/Parameters

4.1 Chromatographic Conditions

Column: Synergi Hydro-RP, 4.0 μ m, 50 mm x 2.0 mm, 80Å, S/N: 549505-6; P/N: 00B-4375-B0 (for F8426, F8426-CI-PAC, F8426-CAc and F8426-PAC).

Column: Gemini C18, 5.0 μ m, 50 mm x 2.0 mm, 110Å, S/N: 339303-2; P/N: 00B-4435-B0 (for F8426-BAC).

Flow rate: 0.500 mL/min.

Run time: 5.10 minutes

Mobile phase A: 0.1% Formic acid in D.I. Water (for F8426, F8426-CI-PAC, F8426-CAc and F8426-PAC)

Mobile phase B: 0.1% Formic acid in acetonitrile (for F8426, F8426-CI-PAC, F8426-CAc and F8426-PAC)

Mobile phase A: 10 mM NH₄HCO₃ in DI water (for F8426-BAC)

Mobile phase B: 10 mM NH₄HCO₃ in ACN:H₂O (v:v, 90:10) (for F8426-BAC)

Elution gradient table:

Time (minutes)	A (%)	B (%)	Flow Rate (mL/min)
0.50	98	2	0.5
2.00	5	95	0.5
4.00	5	95	0.5
4.10	98	2	0.5
5.10	98	2	0.5

4.2 Autosampler Properties

Rinsing Volume (μ L)	400
Injection Volume (μ L)	1.00
Injection Volume (μ L)	10.0 ^a
Sampling Speed (μ L/sec)	5

^a F8426-BAC

4.3 Mass Spectrometer Method Properties

For F8426, F8426-CI-PAC, F8426-CAc and F8426-PAC

Acquisition Duration	5.1 minutes
Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Gas 1 (GS1)	50

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Gas 2 (GS2)	50
Collision Gas (CAD)	10
Curtain Gas (CUR)	25
Ion Spray Voltage (IS)	5500
Temperature (TEM)	500

For F8426-BAc

Acquisition Duration	5.1 minutes
Ionization Mode	ESI
Scan Type	MRM
Polarity	Negative
Gas 1 (GS1)	50
Gas 2 (GS2)	50
Collision Gas (CAD)	10
Curtain Gas (CUR)	25
Ion Spray Voltage (IS)	-4500
Temperature (TEM)	500

4.4 Mass Transitions and Voltages

Analyte	Q1	Q3	Time (msec)	DP	EP	CE	CXP
Carfentrazone-ethyl (F8426)	412	346	150	101	10	25	12
F8426-chloropropionic acid (F8426-CI-PAc)	384	346	150	126	10	27	12
F8426-cinnamic acid (F8426-CAc)	350	312	150	126	10	23	10
F8426-propionic acid (F8426-PAc)	352	314	150	126	10	23	10
F8426-benzoic acid (F8426-BAc)	320	276	150	-70	-10	-14	-7

5.0 Validation Procedures

The method validation was conducted during September 18 – December 19, 2012 at PASC in Princeton, New Jersey. Soil samples were spiked with analytes and extracted by reflux on September 18-20, 2012. Aliquots of the extraction solutions, without clean up by DCM (dichloromethane) partition, were injected directly to evaluate recovery. The results showed acceptable recovery of all analytes. However, the extraction procedure was repeated with the spiked samples and the extraction solutions were further partitioned with DCM on October 17-18, 2012 to be consistent with the original method. The recovery samples fortified concentrations at the LOQ level were below the calibration curve range due to over dilution of the extracted recovery samples with injection solution.

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The recovery samples were then reinjected (on December 19-20, 2012) with less dilution and the recovery data were within the calibration range (reported in Table 6A-6E). Although the redilution and reinjection occurred two months after the initial extraction and partition with DCM, the stability of analytes in DCM was fully supported by the overall acceptable recoveries. Further, the stability of soil extracts in DCM had been established during a 9-month storage interval at room temperature in the previous study (RAN-0270M). The sample extracts in DCM between the 1st and 2nd injections (2 months) were stored under refrigeration conditions at 4°C in this ILV study.

5.1 Standard Solution Preparation

- 5.1.1 Stock Standard Solution preparation: 11.54 mg of carfentrazone-ethyl standard (95.5% purity) was weighed and dissolved with 11.021 mL of ACN to obtain a stock solution of carfentrazone-ethyl with 1.00 mg/mL concentration; 8.04 mg of F8426-cinnamic acid (98.3% purity) was weighed and dissolved with 7.903 mL of ACN to obtain a stock solution of F8426-cinnamic acid with 1.00 mg/mL concentration; 6.50 mg of F8426-chloropropionic acid (98.0% purity) was weighed and dissolved with 6.370 mL of ACN to obtain a stock solution of F8426-chloropropionic acid with 1.00 mg/mL concentration; 10.95 mg of F8426-propionic acid (98.8% purity) was weighed and dissolved with 10.819 mL of ACN to obtain a stock solution of F8426-propionic acid with 1.00 mg/mL concentration; 10.24 mg of F8426-benzoic acid (99.4% purity) was weighed and dissolved with 10.179 mL of ACN to obtain a stock solution of F8426-benzoic acid with 1.00 mg/mL concentration. In a 10-mL volumetric flask, 10.0 µL of each stock solution above was transferred and diluted to the mark with ACN to obtain a mixed stock solution with 1000 ng/mL concentration of each compound. All the above stock solutions were stored at 2-8°C.
- 5.1.2 Calibration Standard Solution preparation: A series of calibration standard solutions were prepared according to **Table 1**, starting from the mixed stock solution containing 1000 ng/mL concentration of each compound.

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Table 1. Calibration Standard Solution Preparation

Standard Solution ID	Source Solution ID	Source Concentration (ng/mL)	Aliquot (mL)	Total Vol. (mL)	Concentration (ng/mL)
Std-1	Mixed Stock	1000	1.00	1.00	1000
Std-2	Mixed Stock	1000	0.750	1.00	750
Std-3	Mixed Stock	1000	0.250	1.00	250
Std-4	Mixed Stock	1000	0.0500	1.00	50.0
Std-5	Mixed Stock	1000	0.0200	1.00	20.0
Std-6	Mixed Stock	1000	0.0100	1.00	10.0
Std-7	Std-6	10.0	0.200	1.00	2.00
Std-8	Std-6	10.0	0.100	1.00	1.00

5.2 Residue Sample Preparation

5.2.1 Fortification: To each of the twelve 500-mL boiling flask, 10.0 g of soil were transferred and fortified with the mixed standard solution as outlined in Table 2. The samples were then shaken and mixed. A boiling flask without soil was used for reagent blank and was not fortified.

Table 2. Soil Sample Fortifications

Sample ID	Soil (g)	Fortification Solution ID	Fortification Solution Concentration (ng/mL)	Fortification Volume (µL)	Fortification Level (ppb)
Reagent Blank	0	N/A	N/A	0	0
Control 1 and 2	10.0	N/A	N/A	0	0
LOQ 1 to 5	10.0	Stock	1000	50	5.00
10xLOQ 1 to 5	10.0	Stock	1000	500	50.0

5.2.2 Extraction

All glassware used during the extraction (including the boiling flasks, bottles, tubes, pasteur pipettes, funnels and syringes etc) were pre-rinsed with generous amount of 0.25N HCl, followed by acetone to remove acid.

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To each fortified soil sample and control/reagent blank sample, 25.0 mL of ACN/D.I.Water (8:2, v:v) was added and the boiling flask was placed on hot plate under a cold water condenser. Reflux the sample for 60 minutes and allow to cool for ~30 minutes. Rinse the condenser with about 1.0 mL of the reflux solvent and collect all the reflux solvent.

Pre-wet the filtering apparatus, including filter paper with the reflux solvent. Carefully filter the sample through a Whatman GF/A filter paper into a glass bottle. Rinse the boiling flask and the filter cake with ~5.0 mL of reflux solvent.

Evaporate the filtrate to a volume of ~4 mL. Bring the sample to ~6 mL with deionized water. Add 125 μ L of 10% H₂SO₄ and 0.5 g sodium chloride. Extract and partition the sample with 10 mL of methylene chloride (DCM) by vigorous vortexing. Let the tube (glass tube) sit on bench top for ~10 min to separate phases. After phase separation, use a glass pasteur pipette to transfer the organic phase to a glass syringe pre-loaded with a thin layer of glass wool and granular sodium sulfate. Collect the flow-through in a glass tube. Extract and partition the sample with another 10 mL of DCM and repeat the collection process. Evaporate the flow-through just to dryness under a gentle stream of nitrogen. Reconstitute the sample with 25.0 mL of ACN/D.I. Water (8:2, v/v).

5.2.3 Modification to the original methods

The following modifications were applied to the methods:

- 1) The quantification method was changed from GC to LC/MS/MS;
- 2) Original chemical derivatization and SPE clean-up steps were eliminated due to the use of highly selective LC/MS/MS technique.

5.3 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.

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The validation set contained at least one reagent blank, two unfortified matrix controls, five matrix control samples fortified at 5.00 ppb as LOQ level and five matrix control samples fortified at 50.0 ppb as 10x LOQ level. The injection sequence is outlined in **Tables 3-3A**.

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Table 3. Injection Sequence

Injection Sequence	Sample Type
1	Solvent Blank
2-3	Matrix Control Blank 1 to 2
4	Reagent Blank
5	Standard Solution 1.00 ng/mL
6	Standard solution 2.00 ng/mL
7-11	Fortified sample extracts LOQ 1-5
12	Standard solution 10.0 ng/mL
13	Standard solution 20.0 ng/mL
14-18	Fortified sample extracts 10x LOQ 1-5
19	Standard solution 50.0 ng/mL
20	Standard solution 250 ng/mL
21	Standard solution 750 ng/mL
22	Standard solution 1000 ng/mL

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Table 3A. Injection Sequence (F8426-BAc)

Injection Sequence	Sample Type
1-2	Solvent Blank
3-4	Matrix Control Blank 1 to 2
5	Reagent Blank
6	Standard Solution 1.00 ng/mL
7	Standard solution 2.00 ng/mL
8-12	Fortified sample extracts LOQ 1-5
13	Standard solution 10.0 ng/mL
14	Standard solution 20.0 ng/mL
15-19	Fortified sample extracts 10x LOQ 1-5
20	Standard solution 50.0 ng/mL
21	Standard solution 250 ng/mL
22	Standard solution 750 ng/mL
23	Standard solution 1000 ng/mL

Note: The injection sequence for F8426-BAc had two solvent blanks while all the other analytes had the sequence as described in Table 3.