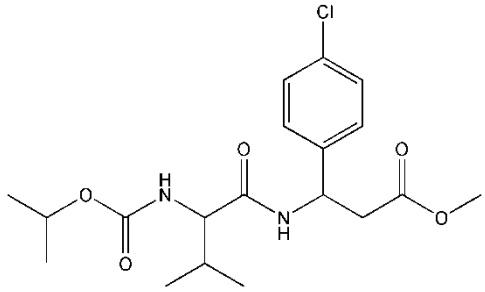
1.0 SUMMARY

The objective of this study phase was to determine and report the residues of valifenalate, valifenalate acid, and *p*-chlorobenzoic acid (PCBA) in application verification spray pads, soil cores, and travel stability samples from Precision Study Management Study PSM-14-02-08, "Terrestrial Field Dissipation of F9170 in Nebraska, USA."

2.0 ANALYTICAL STANDARDS

A summary of the information concerning the analytical standards is listed below which includes structure, chemical name, purity and expiration date. The certificates of analysis (CoA) are included in Appendix A.

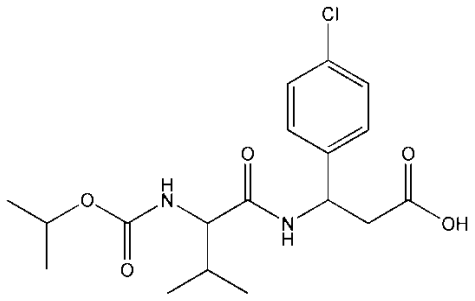
2.1 VALIFENALATE



Valifenalate

Common Name:	Valifenalate
Chemical Name:	Methyl N-(isopropoxycarbonyl)-*L*-valyl-(3*R*,*S*)-2-(4-chlorophenyl)-β-alanine
CAS No.:	283159-90-0
Molecular Formula:	$C_{19}H_{27}ClN_2O_5$
Lot No.:	G019/07 (ID in CoA: IR5885)
Purity:	99.52%
Expiration Date:	Nov 2017
Molecular Weight:	398.88 g/mole
Storage:	Ambient Temperature

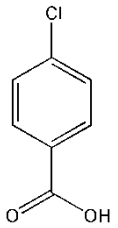
2.2 *VALIFENALATE ACID*



Valifenalate acid

Common Name:	Valifenalate acid
Chemical Name:	*(R,S)*-β-alanine, N-((1-methylethoxy)-*L*-valy-3-(4-chlorophenyl) acid)
CAS No.:	NA
Molecular Formula:	$C_{18}H_{25}ClN_2O_5$
Lot No.:	G029/08 (ID in CoA: IR5839)
Purity:	98.4%
Expiration Date:	Feb 21, 2017
Molecular Weight:	384.86 g/mole
Storage:	Ambient Temperature

2.3 *P-CHLOROBENZOIC ACID (PCBA)*



p-Chlorobenzoic acid (PCBA)

Common Name:	p-Chlorobenzoic acid (PCBA)
Chemical Name:	4-Chlorobenzoic acid
CAS No.:	74-11-3
Molecular Formula:	$C_7H_7ClO_2$
Lot No.:	LC07337V
Purity:	99.2%
Expiration Date:	May 2017
Molecular Weight:	156.57 g/mole
Storage:	Ambient Temperature

3.0 EXPERIMENTAL SECTION

3.1 MATERIALS

3.1.1 Chemicals

Acetic acid, Fisher HPLC Grade
Formic acid, Fisher Optima LC-MS Grade
Hydrochloric acid 1 N solution, Fisher Scientific
Acetone, Fisher Optima Grade
Acetonitrile, Fisher Optima Grade
Methanol, Fisher Optima Grade
Water, Fisher HPLC Grade

3.1.2 Reagents

Extraction solvent: Acetone:0.5 N HCl in water (50:50, v:v)\
Acetone (2000 mL) and 0.5 N HCl in water (2000 mL) were mixed.
(0.5 N HCl in water was prepared by mixing 1 N HCl solution (1000 mL) with HPLC water (1000 mL)

Extraction solvent: Acetone:0.5 N HCl in water (20:80, v:v)
Acetone (800 mL) and 0.5 N HCl in water (3200 mL) were mixed.
(0.5 N HCl in water was prepared by mixing 1 N HCl solution (1600 mL) with HPLC water (1600 mL)

Dilution solvent: methanol -water (50:50) + 0.1% formic acid
Methanol (500 mL), HPLC water (500 mL), and formic acid (1 mL) were mixed.

Dilution solvent: methanol -water (10:90) + 0.1% formic acid
Methanol (100 mL), HPLC water (900 mL), and formic acid (1 mL) were mixed.

HPLC solvent A: 0.1% formic acid in water
HPLC water (1 L) and formic acid (1 mL) were mixed.

HPLC solvent B: 0.1% formic acid in acetonitrile
Acetonitrile (1 L) and formic acid (1 mL) were mixed.

HPLC solvent A: 0.1% acetic acid in water
HPLC water (1 L) and acetic acid (1 mL) were mixed.

HPLC solvent B: 0.1% acetic acid in acetonitrile
Acetonitrile (1 L) and acetic acid (1 mL) were mixed.

3.1.3 Equipment

The following contains a partial list of the equipment used in this study. Any equipment used in this study and not contained in the following list can be found in the appropriate sections of this report.

Analytical electronic balance with 0.1-mg readability
Eppendorf micropipettes: 20-200 µL, and 100-1000 µL
Glassware: Assorted beakers, bottles, graduated cylinders, pipettes, etc., which are routinely used for residue analysis.

3.1.4 Preparation of Standard Solutions and Calibration Solutions

The following standard solutions and calibration solutions were prepared by mixing of the following stock solutions, followed by serial dilution with methanol-water (50:50, v:v) + 0.1% formic acid or methanol-water (10:90, v:v) + 0.1% formic acid in "Class A" volumetric flasks, as detailed in Table 1 below. All solutions were stored in a freezer (~-20 ºC) when not in use.

- 1005 µg/mL Valifenalate in acetone stock solution (purity corrected)
- 1004 µg/mL Valifenalate acid in acetone stock solution (purity corrected)
- 1002 µg/mL p-Chlorobenzoic acid (PCBA) in acetone stock solution (purity corrected)

Table 1: Preparation Scheme for Standard Solutions and Calibration Solutions

Standard solutions in methanol -water (50:50) + 0.1% formic acid			
Solution used	**Volume taken (mL)**	**Final volume (mL)**	**Nominal concentration (µg/mL)**
1005 µg/mL Valifenalate	0.249	50	5
1004 µg/mL Valifenalate Acid	0.249		
1002 µg/mL PCBA	0.248		
5 µg/mL	5	25	1 (1000 ng/mL)
5 µg/mL	5	50	0.5
Calibration solutions in methanol -water (10:90) + 0.1% formic acid			
Solution used	**Volume taken (mL)**	**Final volume (mL)**	**Nominal concentration (ng/mL)**
1000 ng/mL	5	50	100
1000 ng/mL	2.5	50	50
1000 ng/mL	1	50	20
100 ng/mL	5	50	10
50 ng/mL	5	50	5
20 ng/mL	5	50	2
10 ng/mL	5	50	1
5 ng/mL	5	50	0.5
2 ng/mL	5	50	0.2
1 ng/mL	5	50	0.1
0.5 ng/mL	5	50	0.05

3.1.5 Preparation of Travel Stability Kits

Separate travel stability kits were prepared for valifenalate, valifenalate acid, and PCBA. Each travel stability kit consisted of twelve 10-g samples of untreated control soil (UTC-SC-1-CTR-6), three 1-mL aliquots of methanol-water (50:50) + 0.1% formic acid, and nine 1-mL aliquots of 0.5-µg/mL valifenalate, valifenalate acid, or PCBA (as appropriate) in methanol-water (50:50) + 0.1% formic acid. The travel stability kits were shipped frozen on dry ice to the field site for spiking.

3.2 METHOD VALIDATION AND CONCURRENT SOIL SAMPLES

The validation of the Eurofins Method # RA034 with some modification (both soil aliquot and extraction solvent volume were doubled, and HPLC solvents and gradient were modified) was conducted prior to sample analysis. Method validation samples consisted of 2 unfortified Nebraska (NE) control soil samples, 3 NE soil samples spiked at the target LOQ of 0.005 ppm for valifenalate and valifenalate acid and 0.01 ppm for PCBA, and 3 NE soil samples spiked at 0.05 ppm for valifenalate and valifenalate acid and 0.1 ppm for PCBA (10x the LOQ). Once validated, the method was used for extraction and quantification of residues of valifenalate, valifenalate acid, and PCBA in soil in F9170 terrestrial field dissipation (TFD) studies.

3.2.1 Preparation of Fortification Solutions

The following fortification solutions were prepared by mixing and dilution of the above stock solutions with MeOH-water (50:50, v/v+ 0.1% formic acid) in "Class A" volumetric flasks, as detailed below:

Solution used	Volume taken (mL)	Final volume (mL)	Nominal concentration (µg/mL)
1005 µg/mL Valifenalate	0.124	50	Valifenalate, valifenalate acid, PCBA 2.5/2.5/5
1004 µg/mL Valifenalate Acid	0.124		
1002 µg/mL PCBA	0.248		
2.5/2.5/5 µg/mL	5	50	Valifenalate, valifenalate acid, PCBA 0.25/0.25/0.5

3.2.2 Fortification of Method Validation and Soil Concurrent Samples

The method validation samples and soil concurrent samples (~10 g each) were fortified as detailed below:

	Control Soil (g)	Fortification Solution	Amount
Control	10	Methanol-water (50:50) + 0.1% formic acid	200 µL
LOQ	10	Valifenalate, valifenalate acid, PCBA 0.25/0.25/0.5 µg/mL*	200 µL
10xLOQ	10	Valifenalate, valifenalate acid, PCBA 2.5/2.5/5 µg/mL*	200 µL

**in methanol -water (50:50) + 0.1% formic acid*

The samples were extracted and analyzed according to the procedures detailed in Sections 3.4.2 and 3.4.3.

3.3 VERIFICATION PAD ANALYSIS

Jars containing the verification pads (set "A") + 100 mL of acetonitrile were received from the field site and stored frozen (~-20 ºC). Aliquots (100 µL) of each verification pad extract were diluted 1000-fold with methanol-water (10:90) + 0.1% formic acid and analyzed for valifenalate by LC-MS/MS. The "A" set of verification pads was analyzed. The "B" set of verification pads

(in Petri dish) was not analyzed, because acceptable data were obtained for the "A" set. The "B" set of verification pads was stored frozen at ~ -20 ºC. Also, tank mixes were not analyzed because acceptable data were obtained for the spray pad analysis.

3.4 SOIL CORE ANALYSIS

3.4.1 Homogenization and Determination of Moisture Content

The soil core samples were received frozen from the field site and stored frozen (~ -20 ºC) until homogenization. Travel stability samples were received with the sample shipment for Interval 17.

The soil core samples were homogenized with dry ice in a Hobart 8181D. The dry ice was permitted to sublime before samples were weighed for analysis. The soil moisture content was determined by weighing three aliquots (approximately ten grams each) of soil in aluminum weighing dishes and recording the dry weight of the soil after drying overnight in an oven set at approximately 100 ºC. The percent moisture loss was used to calculate dry weight residues of all samples and was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{initial sample weight as received - sample weight after drying}}{\text{initial sample weight as received}} \text{ x } 100$$

3.4.2 Soil Extraction

Residues of valifenalate, valifenalate acid, and PCBA were extracted from ten grams of soil using the procedure described below and quantified for valifenalate, valifenalate acid, and PCBA by LC-MS/MS as indicated in Section 3.4.3.

1. Aliquots (approximately 10 g each) of soil were weighed into 50-mL polypropylene centrifuge tubes. Concurrent recovery samples were fortified with the appropriate amount of valifenalate, valifenalate acid, and PCBA at this step.
2. Acetone/0.5 N HCl in water (50:50) (20 mL) was added to each sample.
3. The samples were shaken using a Burrell wrist-action shaker for 30 minutes.
4. The samples were centrifuged for 10 minutes at ~3000 rpm.
5. The supernatant was transferred to a new 50-mL polypropylene centrifuge tube.
6. The solid residue was re-extracted with 20 mL of acetone/0.5 N HCl in water (20:80), as in steps 3 to 5, combining the supernatants in the 50-mL polypropylene centrifuge tube.
7. The combined extracts were centrifuged for 10 minutes at ~3000 rpm.
8. A 10-mL aliquot of the combined extracts was transferred to a 15-mL polypropylene centrifuge tube and concentrated to ~6.5 mL in a 40 ºC water bath under a nitrogen purge.
9. The sample was brought back to 10 mL with methanol/water (50:50) + 0.1% formic acid.
10. A 1-mL aliquot of the sample was diluted 10x with methanol/water (10:90) + 0.1% formic acid.

11. Samples were transferred to autosampler vials for LC-MS/MS analysis for valifenalate and valifenalate acid and PCBA.

3.4.3 LC-MS/MS Analysis of Soil Extracts

Valifenalate and Valifenalate Acid

HPLC:
Column: Analytical Advantage ARMOR C18, 5 µm, 100 x 2.1 mm, P/N ADV7009
Column Temperature: 40 ºC
Injection Volume: 20-40 µL for soil analysis, 10 µL for verification pad analysis
Solvent System:
Solvent A = 0.1% formic acid in water
Solvent B = 0.1% formic acid in acetonitrile

Solvent program:

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.0	0.8	80	20
1.5	0.8	80	20
1.7	0.8	5	95
3.0	0.8	5	95
3.1	0.8	80	20
5.0	0.8	80	20

The LC flow is diverted to the MS between 1.0 and 3.9 min and to waste between 0.0 and 1.0 min and between 3.9 and 5.0 min.
Retention times: Valifenalate ~3.3 min, Valifenalate acid ~3.2 min;

Mass Spectrometer: SCIEX API 4000

Scan Type:	MRM
Polarity:	Positive
Ion Source:	Turbo Spray
Resolution Q1	Unit
Resolution Q3	Unit
Ion Source Gas 1 (GS1):	50 psi
Ion Source Gas 2 (GS2):	70 psi
Curtain Gas (CUR):	20 psi
Collision Gas (CAD):	6 psi
IonSpray Voltage (IS):	5500 V
Temperature (TEM):	400 ºC
Entrance Potential (EP):	10 V
Collision Gas Exit Potential (CXP):	12 V

MRM Transition

MRM Method	Analyte ID	Q1 Mass (amu)	Q3 Mass (amu)	Declustering Potential (DP)	Collision Energy (CE)	Dwell Time (msec)
Primary	Valifenalate	399	155	66 V	47 V	100
Confirmatory	Valifenalate C1	399	144	66 V	21 V	100
Confirmatory	Valifenalate C2	399	116	66 V	33 V	100
Primary	Valifenalate acid	385	116	51 V	31 V	100
Confirmatory	Valifenalate acid C1	385	186	51 V	17 V	100
Confirmatory	Valifenalate acid C2	385	144	51 V	21 V	100

Only primary MRM transition data were reported in this report. Confirmatory MRM transition data were for information purposes only.

Calibration Standards for Analysis of Valifenalate and Valifenalate Acid

For analysis of valifenalate and valifenalate acid in soil samples, a series of calibration standards containing a mixture of valifenalate, valifenalate acid, and PCBA at 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 ng/mL was prepared to quantify the observed residues in the soil samples. For analysis of valifenalate in verification pad samples, a series of calibration standards containing a mixture of valifenalate, valifenalate acid, and PCBA at 0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/mL was prepared to quantify the observed residues in the verification pad samples.

PCBA

HPLC:

Column: Analytical Advantage ARMOR C18, 5 µm, 100 x 2.1 mm, P/N ADV7009
Column Temperature: 40 ºC
Injection Volume: 50 µL
Solvent System:
Solvent A = 0.1% acetic acid in water
Solvent B = 0.1% acetic acid in acetonitrile
Solvent Program:

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.0	0.8	90	10
2.5	0.8	10	90
3.0	0.8	10	90
3.1	0.8	90	10
5.0	0.8	90	10

The LC flow is diverted to the MS between 1.0 and 4.0 min and to waste between 0.0 and 1.0 min and between 4.0 and 5.0 min.
Retention times: PCBA ~2.6 min

Mass Spectrometer: SCIEX API 4000

Scan Type:	MRM
Polarity:	Negative
Ion Source:	Turbo Spray
Resolution Q1	Unit
Resolution Q3	Unit
Ion Source Gas 1 (GS1):	60 psi
Ion Source Gas 2 (GS2):	50 psi
Curtain Gas (CUR):	19 psi
Collision Gas (CAD):	8 psi
IonSpray Voltage (IS):	-4500 V
Temperature (TEM):	550º C
Declustering Potential (DP)	-30 V
Entrance Potential (EP):	-10 V

MRM Transition

MRM Method	Analyte ID	Q1 Mass (amu)	Q3 Mass (amu)	Collision Energy (CE)	Collision Gas Exit Potential (CXP)	Dwell Time (msec)
Primary	PCBA	155	111	-16 V	-7 V	500
Confirmatory	PCBA-C	155	35	-52 V	-3 V	500

Only primary MRM transition data were reported in this report. Confirmatory MRM transition data were for information purposes only.

Calibration Standards for Analysis of PCBA

For analysis of PCBA, a series of calibration standards containing a mixture of valifenalate, valifenalate acid, and PCBA at 0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/mL was prepared to quantify the observed residues in the soil samples.

3.5 STATISTICAL ANALYSIS AND GENERAL CALCULATIONS

Descriptive statistics (mean and SD) were calculated using Microsoft® Excel 2010.

Quantification of residues was made by injecting with the samples a series of calibration standards. The peak area measured by the MS/MS detector was collected by Analyst™ version 1.4.2. Calibration plots were generated by Analyst™ version 1.4.2 using the peak area responses of the external calibration standards injected with the samples. The sample concentration in ng/mL was determined automatically by Analyst™ version 1.4.2 from the peak area response of the sample and the slope and intercept of the linear plot of the standards.

The raw data generated by Analyst™ were transferred to a spreadsheet program, Microsoft® Excel 2010, where appropriate calculations of residue levels in spray pads and soil were performed. Calculations were made based on the full precision of the program. Rounded values were reported in the tables and text for presentation purposes only, but in the internal spreadsheet

program for calculation the values were not rounded. Thus, hand calculations to obtain values given in the tables of this report may differ slightly from those presented due to rounding.

Example calculations are provided in Appendix B.

Calculation of Soil Residues in ppb

The concentrations of analytes (valifenalate, valifenalate acid, and PCBA) in samples were calculated based on the linear calibration curves generated with the analytical set:

Linear regression formula from calibration curve y = mx + b

$$x \text{ (analyte in ng/mL)} = \frac{y - b}{m}$$

Where y = Sample peak area

b = Calibration curve intercept

m = Calibration curve slope

$$\text{Sample concentration (ng/mL)} = \frac{\text{Sample peak area - intercept}}{\text{Slope}}$$

The analyte concentrations in ppb in each sample were calculated as follows

$$\text{ppb analyte (wet weight basis)} = \frac{\text{Sample Conc. (ng/mL) x Extract Vol. (mL) x Dilution factor}}{\text{Sample weight (grams)}}$$

where ng/g is equivalent to µg/kg and ppb.

An example calculation for the concentrations of valifenalate for sample NE_T1-SC-9-SPA-6 (Interval 9, Subplot A, 0-6" soil core. Table 9, Figure 21) is shown below:

The calibration curve equation was y = 54800x – 0.922 (r = 0.9999):

$$\text{ng/mL valifenalate} = \frac{32700 + 0.922}{54800} = 0.597 \text{ ng/mL}$$

$$\text{ppb valifenalate} = \frac{0.597 \text{ ng/mL x } 40 \text{ mL x } 10}{10.00 \text{ grams}} = 23.88 \text{ ppb}$$

Calculation of Soil Wet Weight Residue to Soil Dry Weight Residue

The concentrations of analytes in soils on a dry weight basis (in ppb) were calculated from the concentrations on a wet weight basis (as received, in ppb):

$$\text{ppb (dry weight basis)} = \frac{\text{ppb (wet weight basis)}}{1 - (\% \text{ Moisture}/100)}$$

$$\% \text{ Moisture} = \frac{\text{weight of wet soil - weight of dry soil after drying}}{\text{weight of wet soil}} \times 100$$

An example calculation for the concentrations of valifenalate for sample NE_T1-SC-9-SPA-6 (% moisture was 10.8%) is shown below:

$$\text{ppb Valifenalate} = \frac{23.88 \text{ ppb}}{1 - (10.8/100)} = 26.77 \text{ ppb}$$

Calculation of Soil Concurrent Sample Recovery

The recoveries of each analyte from fortified samples were calculated as follows:

$$\text{ppb (wet weight basis)} = \frac{\text{Sample Conc. (ng/mL) x Extract Vol. (mL) x Dilution factor}}{\text{Sample weight (grams)}}$$

where ng/g is equivalent to ppb.

$$\text{Percent Recovery} = \frac{\text{Conc. of Fortified Sample (ppb) - Conc. of Control (ppb)}}{\text{Fortification Level (ppb)}} \times 100\%$$

An example calculation for the recovery of valifenalate (0.005 ppm or 5 ppb fortification associated with the analysis of Interval 9, Day-1 treated samples) is shown below:

The ppb valifenalate (wet weight basis) was calculated as shown:

$$\text{ppb valifenalate} = \frac{0.09811 \text{ ng/mL x } 40 \text{ mL x } 10}{10.01 \text{ grams}} = 3.92 \text{ ppb}$$

The percent recovery of valifenalate was calculated as follows:

$$\text{Valifenalate Percent Recovery} = \frac{3.92 \text{ ppb} - 0 \text{ ppb}}{5 \text{ ppb}} \times 100\% = 78.4\%$$