

**Analytical method for valifenalate, valifenalate acid and p-chlorobenzoic acid in water**

**Reports:** ECM: EPA MRID No.: MRID 49807180. Ferguson, L-J.C. 2015. ANALYTICAL METHOD REPORT – Method Validation- Determination of Residues of Valifenalate and its Metabolites Valifenalate acid and p-Chlorobenzoic Acid in Water. Ricerca Document No.: 032663-1. Ricerca Study No.: 032663. FMC Tracking No.: 2014RES-VAL1464. Report prepared by Ricerca Biosciences, LLC, Concord, Ohio, and sponsored and submitted by FMC Corporation, Agricultural Products Group, Ewing, New Jersey; 108 pages. Final report issued July 16, 2015.

ILV: EPA MRID No.: MRID 49807179. Shanmugan, S. 2015. Independent Laboratory Validation (ILV) of an Analytical Method for the Determination of Valifenalate and Two Metabolites (Valifenalate acid and p-Chlorobenzoic Acid) in Water. FMC Tracking No.: 2015RES-VAL2062. Battelle ID: 100066692. Report prepared by Battelle, Norwell, Massachusetts, and sponsored and submitted by FMC Corporation, Agricultural Products Group, Ewing, New Jersey; 64 pages. Final report issued November 17, 2015.

**Document No.:** MRIDs 49807179 & 49807180

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 49807180). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The Authenticity statement was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, which are compatible with OECD GLP (pp. 3, 5 of MRID 49807179). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-5). The statement of authenticity was included with the quality assurance statement.

**Classification:** This analytical method is classified as Supplemental. The specificity of the method was not supported by the ILV representative chromatograms of valifenalate or the ECM representative chromatograms of p-chlorobenzoic acid due to matrix interferences. No citations or justifications for chosen LODs or LOQs are provided in either the ECM or ILV.

**PC Code:** 128200

**EFED Final Reviewer:** Sarah C. Hafner, Ph.D.,  
Chemist

Signature:  
Date: 5/11/19

**CDM/CSS- Dynamac JV Reviewers:** Lisa Muto,  
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*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## Executive Summary

The analytical method, Ricerca Document No. 032663-1, is designed for the quantitative determination of valifenalate, valifenalate acid, and p-chlorobenzoic acid in water at the stated LOQ of 0.1 µg/L. The LOQ is less than the lowest toxicological level of concern in water. Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid, and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters. The ECM and ILV matrices were different surface (pond) waters. The analytes were identified using two ion transitions in the ECM and ILV. All ILV data regarding linearity, repeatability, accuracy, precision, and specificity were satisfactory for all three analytes at the LOQ, except that the specificity of the method was not supported by the representative chromatograms of valifenalate. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for all three analytes, except that the specificity of the method was not supported by the representative chromatograms of p-chlorobenzoic acid. The LOD of the method for valifenalate and valifenalate acid differed between the ECM and ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Valifenalate	49807180 <sup>1</sup>	49807179 <sup>2</sup>		Water	16/07/2015	FMC Corporation	LC/MS/MS	0.1 µg/L
Valifenalate acid								
p-Chlorobenzoic acid								

<sup>1</sup> In the ECM, the surface water (pH 7.8, hardness 98 mg equivalent CaCO<sub>3</sub>/L, 124 ppm total dissolved solids) was obtained from Smokey Oaks Pond, Penny Minor, North Bloomfield, Trumbull County, Ohio (41° 49'54.46N 80° 74'33.7W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix A, Figure A-1, p. 33 of MRID 49807180).

<sup>2</sup> In the ILV, the surface water (pH 7.2, hardness 37 mg equivalent CaCO<sub>3</sub>/L, 276 ppm total dissolved solids) was obtained from Jacob's Pond (49° 09'33.9N 70° 50'51.5W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 2, p. 64 of MRID 49807179). The source location was not further described.

## I. Principle of the Method

For the analysis of valifenalate and its metabolites, the water (100 mL aliquots) was fortified then mixed with 5 mL of 1 N HCl and applied to a Waters OASIS solid phase extraction (SPE) cartridge (HLB, 6 cc, 500 mg; pre-conditioned with one 5 mL aliquot each of methanol then water; p. 17 of MRID 49807180). The sample flask was rinsed with a mixture of 20 mL water and 1 mL 1 N HCl; the rinse was applied to the cartridge. The eluates were discarded, and the analytes were eluted with 5 mL methanol into 15-mL polypropylene centrifuge tube. The eluate was diluted to 10 mL using HPLC water, and an aliquot was transferred to an HPLC vial for LC-MS/MS analysis.

For valifenalate and valifenalate acid identification, two Shimadzu LC20-AD HPLC pumps and a Shimadzu SIL-HTA autosampler coupled with a SCIEX API 4000 Mass Spectrometer using multiple reaction monitoring (MRM) in positive mode (400°C) was used (pp. 18-20 of MRID 49807180). The following LC conditions were employed: ARMOR C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-1.5 min. 80:20, 1.7-3.0 min. 5:95, 3.1-5.0 min. 80:20] and injection volume of 10 µL. The transitions used for quantitation were m/z 399→155 for valifenalate and m/z 385→116 for valifenalate acid. The transitions used for confirmation were m/z 399→116 for valifenalate and m/z 385→144 for valifenalate acid.

Retention times were *ca.* 3.3 and 3.2 minutes for valifenalate and valifenalate acid, respectively. A separate chromatographic method was used to separate p-chlorobenzoic acid using the same type LC-MS/MS system under negative ionization and using multiple reaction monitoring (MRM; 550°C) mode. The following LC conditions were employed: ARMOR C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0 min. 90:10, 2.5-3.0 min. 10:90, 3.1-5.0 min. 90:10] and injection volume of 20 µL. Mass transition m/z 155→111 was used for quantitation, and m/z 155→35 was monitored for confirmation. Retention time was *ca.* 2.6 minutes for p-chlorobenzoic acid.

The ILV performed the ECM method as written, except for the use of 1 mL (instead of 5 mL) of 1 N HCl to acidify samples prior to SPE, a modified calibration concentration range, preparation of only one control sample per sample set, and insignificant modifications to the analytical equipment and parameters (pp. 15, 20-23, 27-28 of MRID 49807179). The LC-MS/MS instrument and parameters were similar to those of the ECM. Samples were analyzed for valifenalate, valifenalate acid, and p-chlorobenzoic acid using a Shimadzu LC2080 UHPLC coupled with an Applied Biosystems MDS Sciex API 6500 Linear Ion Trap Mass Spectrometer (550°C). A Thermo Betasil C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C) was used for valifenalate and valifenalate acid analysis. The other LC conditions were the same as those reported in the ECM. The two monitored ion pair transitions were the same as those of the ECM. Reported retention times were *ca.* 2.2-2.4 for valifenalate and valifenalate acid and *ca.* 2.08-2.14 for p-chlorobenzoic acid. No other method modifications were reported.

In the ECM and ILV, the validated Limit of Quantification (LOQ) was 0.1 µg/L for residues of valifenalate, valifenalate acid, and p-chlorobenzoic acid in water (pp. 11, 27 of MRID 49807180; pp. 10, 12, 26 of MRID 49807179). For residues of valifenalate and valifenalate acid, the method Limit of Detection (LOD) in water was 0.02 µg/L in the ECM and 0.025 µg/L in the ILV. In the ECM and ILV, the method LOD was 0.02 µg/L for residues of p-chlorobenzoic acid in water.

## II. Recovery Findings

ECM (MRID 49807180): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis valifenalate, valifenalate acid, and p-chlorobenzoic acid at fortification levels of 0.1  $\mu\text{g/L}$  (LOQ) and 1  $\mu\text{g/L}$  (10 $\times$ LOQ) in one water matrix (n = 5; Tables 6-8, pp. 24-26; DER Attachment 2). Two ion pair transitions were monitored for valifenalate, valifenalate acid and p-chlorobenzoic acid using LC-MS/MS in positive or negative mode; the quantification and confirmation ion data was comparable. Recoveries for the quantification ion transition of p-chlorobenzoic acid were corrected for residues quantified in the controls since they were  $>30\%$  of the LOQ (36.9% and 37.5% of the LOQ; p. 22; Table 8, p. 26). The surface water (pH 7.8, hardness 98 mg equivalent  $\text{CaCO}_3/\text{L}$ , 124 ppm total dissolved solids) was obtained from Smokey Oaks Pond, Penny Minor, North Bloomfield, Trumbull County, Ohio (41° 49'54.46N 80° 74'33.37W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix A, Figure A-1, p. 33).

ILV (MRID 49807179): Mean recoveries and RSDs were within guidelines for analysis of valifenalate and valifenalate acid at the fortification level of 0.1  $\mu\text{g/L}$  (LOQ; n = 6) and 1  $\mu\text{g/L}$  (10 $\times$ LOQ; n = 5) in one water matrix. The recovery results for the 10 $\times$ LOQ fortifications only included five samples in the the RSDs of the fortifications at 1  $\mu\text{g/L}$  due to sample spillage reported by the study author. As a result, the study author excluded one sample from the statistics presented in the study report (Tables 2-3, pp. 47-48). Mean recoveries and RSDs were within guidelines for analysis of p-chlorobenzoic acid at fortification levels of 0.1  $\mu\text{g/L}$  (LOQ; n = 6) and 1  $\mu\text{g/L}$  (10 $\times$ LOQ; n = 5) in one water matrix (Table 4, p. 49). The recovery results for the 10 $\times$ LOQ fortifications of p-chlorobenzoic acid only included five samples; the recovery for the sixth sample was reported as "N/A" and has the same sample ID as the spilled sample. Two ion pair transitions were monitored for valifenalate, valifenalate acid, and p-chlorobenzoic acid using LC-MS/MS in positive or negative mode; the quantification and confirmation ion data was comparable. The surface water (pH 7.2, hardness 37 mg equivalent  $\text{CaCO}_3/\text{L}$ , 276 ppm total dissolved solids) was obtained from Jacob's Pond (49° 09'33.9N 70° 50'51.5W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 2, p. 64). The source location was not further described. Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid, and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters (pp. 12, 20-23, 26-28).

**Table 2. Initial Validation Method Recoveries for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Water**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>1</sup>	Relative Standard Deviation (%)
<b>Surface (Pond) Water<sup>2</sup></b>						
Quantitation ion <sup>3</sup>						
Valifenalate	0.1 (LOQ)	5	111-117	114	3	2.3
	1	5	112-115	114	1	1.1
Valifenalate acid	0.1 (LOQ)	5	88.0-93.3	89.4	2	2.5
	1	5	84.7-86.6	85.7	1	1.0
p-Chlorobenzoic acid <sup>4</sup>	0.1 (LOQ)	5	79.2-93.1	84.6	5	6.3
	1	5	78.4-82.5	80.3	2	2.2
Confirmation ion <sup>3</sup>						
Valifenalate	0.1 (LOQ)	5	108-114	111	2	2.2
	1	5	113-116	114	1	1.1
Valifenalate acid	0.1 (LOQ)	5	84.7-95.7	91.2	4	4.7
	1	5	87.2-92.2	89.3	2	2.2
p-Chlorobenzoic acid	0.1 (LOQ)	5	88.3-106	97.9	7	6.8
	1	5	77.4-84.4	81.8	8	3.5

Data (recovery results were corrected when residues quantified in the controls were >30% of the LOQ; pp. 20-22) were obtained from Tables 6-8, pp. 24-26 of MRID 49807180 and DER Attachment 2.

1 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

2 The surface water (pH 7.8, hardness 98 mg equivalent CaCO<sub>3</sub>/L, 124 ppm total dissolved solids) was obtained from Smokey Oaks Pond, Penny Minor, North Bloomfield, Trumbull County, Ohio (41° 495446N 80° 747337W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix A, Figure A-1, p. 33).

3 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 399→155 and m/z 399→116 for valifenalate; m/z 385→116 and m/z 385→144 for valifenalate acid; and m/z 155→111 and m/z 155→35 for p-chlorobenzoic acid.

4 Recoveries for the quantification ion transition of p-chlorobenzoic acid were corrected for residues quantified in the controls since they were >30% of the LOQ (36.9% and 37.5% of the LOQ; p. 22; Table 8, p. 26).

**Table 3. Independent Validation Method Recoveries for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Water**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>1</sup>	Relative Standard Deviation (%)
<b>Surface (Pond) Water<sup>2</sup></b>						
Quantitation ion <sup>3</sup>						
Valifenalate	0.1 (LOQ)	6	67-95	78	10	12
	1.0	5 <sup>4</sup>	84-96	90	5	5
Valifenalate acid	0.1 (LOQ)	6	83-93	88	3	4
	1.0	5 <sup>4</sup>	94-101	98	3	3
p-Chlorobenzoic acid	0.1 (LOQ)	6	83-114	102	11	11
	1.0	5 <sup>5</sup>	98-105	102	3	3
Confirmation ion <sup>3</sup>						
Valifenalate	0.1 (LOQ)	6	76-96	84	7	9
	1.0	5 <sup>4</sup>	85-97	89	5	6
Valifenalate acid	0.1 (LOQ)	6	85-95	88	4	5
	1.0	5 <sup>4</sup>	93-102	97	3	3
p-Chlorobenzoic acid	0.1 (LOQ)	6	86-122	106	13	12
	1.0	5 <sup>5</sup>	100-106	104	2	2

Data (uncorrected recovery results; p. 24) were obtained from Tables 2-4, pp. 47-49 of MRID 49807179 and DER Attachment 2.

1 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

2 The surface water (pH 7.2, hardness 37 mg equivalent CaCO<sub>3</sub>/L, 276 ppm total dissolved solids) was obtained from Jacob's Pond (49° 09'33.9N 70° 50'51.5W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 2, p. 64). The source location was not further described.

3 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 399→155 and m/z 399→116 for valifenalate; m/z 385→116 and m/z 385→144 for valifenalate acid; and m/z 155→111 and m/z 155→35 for p-chlorobenzoic acid.

4 The recovery results for only five samples is reported as the sixth sample was spilled during sample processing (Tables 2-3, pp. 47-48).

5 The recovery results for only five samples were reported; the recovery for the sixth sample was reported as "N/A" (Table 4, p. 49).

### III. Method Characteristics

In the ECM and ILV, the validated LOQ was 0.1 µg/L for residues of valifenalate, valifenalate acid, and p-chlorobenzoic acid in water (pp. 11, 27 of MRID 49807180; pp. 10, 12, 26 of MRID 49807179). For residues of valifenalate and valifenalate acid, the method LOD in water was 0.02 µg/L in the ECM and 0.025 µg/L in the ILV. In the ECM and ILV, the method LOD was 0.02 µg/L for residues of p-chlorobenzoic acid in water. In the ECM, the LOD was defined as the concentration of the lowest linearity calibrant injected. In the ILV, the LODs were set at 25% of the LOQ for valifenalate and valifenalate acid and 20% of the LOQ for p-chlorobenzoic acid. No calculations, justifications, or comparisons to background noise were provided for the LOQs and LODs in the ECM or ILV.

**Table 4. Method Characteristics for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Water**

		Valifenalate	Valifenalate acid	p-Chlorobenzoic acid
Limit of Quantitation (LOQ)	ECM	0.1 µg/L		
	ILV			
Limit of Detection (LOD)	ECM	0.02 g/L		
	ILV	0.025 µg/L		0.02 µg/L
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	ECM	$r^2 = 0.9996$ (Q & C) $r^2 = 0.99$ (C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9990$ (C)
		0.2-20 ng/mL		
	ILV	$r^2 = 0.99962$ (Q) $r^2 = 0.99902$ (C)	$r^2 = 0.99734$ (Q) $r^2 = 0.99878$ (C)	$r^2 = 0.99976$ (Q) $r^2 = 0.99980$ (C)
		0.25-15 ng/mL		0.20-15 ng/mL
Repeatable	ECM <sup>2</sup>	Yes at LOQ and 10×LOQ. (characterized surface water used)		Yes at LOQ and 10×LOQ; however, Q recoveries corrected for residues quantified in the controls (characterized surface water used)
	ILV <sup>3,4</sup>	Yes at LOQ and 10×LOQ. (characterized surface water used)	Yes at LOQ and 10×LOQ. (characterized surface water used)	Yes at LOQ and 10×LOQ. (characterized surface water used)
Reproducible		Yes at LOQ and 10×LOQ.		Yes at LOQ and 10×LOQ.
Specific	ECM	Yes, no matrix interferences were quantified, but some minor baseline noise was observed around the analyte peak.		<b>No</b> , matrix interferences at the analyte retention time were quantified as <i>ca.</i> 37% of the LOQ (Q).
	ILV	<b>No</b> , matrix interferences were <i>ca.</i> 23% of the LOQ (Q; based on peak height). Baseline noise was observed around the Q analyte peak which interfered with peak attenuation and integration. <sup>5</sup>	Yes, matrix interferences were <i>ca.</i> 6% of the LOQ (Q; based on peak height). Some minor baseline noise was observed around the analyte peak.	Yes, matrix interferences were <i>ca.</i> 6-8% of the LOQ (based on peak height). Some minor baseline noise was observed around the analyte peak.

Data were obtained from pp. 11, 22, 27; Tables 6-8, pp. 24-26 (recovery results); Appendix C, Figures C-1 to C-3, pp. 48-50 and Figures C-22 to C-24, pp. 69-71 (calibration curve); Appendix C, Figures C-4 to C-21, pp. 51-68 and Figures C-25 to C-42, pp. 72-89 (chromatograms) of MRID 49807180; pp. 10, 12, 25-27; Tables 2-4, pp. 47-49 (recovery results); Figures 7-9, pp. 35-37 (calibration curves); Figures 13A-15B, pp. 41-46 (chromatograms) of MRID 49807179. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

1 Correlation coefficients ( $r^2$ ) were reviewer-calculated based on  $r$  values (1/x weighted linear regression analysis) reported in the study report (Appendix C, Figures C-1 to C-3, pp. 48-50 and Figures C-22 to C-24, pp. 69-71 of MRID 49807180; Figures 7-9, pp. 35-37 of MRID 49807179; DER Attachment 2). Matrix-matched solutions were used in the ILV (p. 27 of MRID 49807179).

2 In the ECM, the surface water (pH 7.8, hardness 98 mg equivalent  $\text{CaCO}_3/\text{L}$ , 124 ppm total dissolved solids) was obtained from Smokey Oaks Pond, Penny Minor, North Bloomfield, Trumbull County, Ohio (41° 49'54.6N 80° 74'33.7W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix A, Figure A-1, p. 33 of MRID 49807180).

3 In the ILV, the surface water (pH 7.2, hardness 37 mg equivalent  $\text{CaCO}_3/\text{L}$ , 276 ppm total dissolved solids) was obtained from Jacob's Pond (49° 09'33.9N 70° 50'51.5W) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 2, p. 64 of MRID 49807179). The source location was not further described.

4 Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters (pp. 12, 20-23, 26-28 of MRID 49807179).

5 Based on Figure 13A, p. 41 of MRID 49807179.

#### IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method was not supported by the ILV representative chromatograms of the quantitation ion transition of valifenalate due to significant matrix interferences (*ca.* 23% of the LOQ; >LOD) and baseline noise which interfered with peak attenuation and integration (Figure 13A, p. 41 of MRID 49807179).
2. The specificity of the method was not supported by the ECM representative chromatograms of the quantitation ion transition of p-chlorobenzoic acid due to significant matrix interferences at the analyte retention time which were quantified as *ca.* 37% of the LOQ (Table 8, p. 26; Appendix C, Figures C-19 to C-21, pp. 66-68 of MRID 49807180). Additionally, residue recoveries for the quantification ion transition of p-chlorobenzoic acid were corrected for residues quantified in the controls since they were >30% of the LOQ.
3. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 27 of MRID 49807180; pp. 10, 12, 26 of MRID 49807179). In the ECM, the LOD was defined as the concentration of the lowest linearity calibrant injected. In the ILV, the LODs were set at 25% of the LOQ for valifenalate and valifenalate acid and 20% of the LOQ for p-chlorobenzoic acid. No calculations, justifications, or comparisons to background noise were provided for the LOQs and LODs in the ECM or ILV. The LOD of valifenalate and valifenalate acid differed slightly between the ILV and ECM.
4. Matrix effects were evaluated in the ILV (pp. 25, 27; Tables 11-13, pp. 56-57 of MRID 49807179). Matrix effects were substantial ( $\geq 20\%$ ) for both transitions of the three analytes. Matrix-matched calibration standards were used.
5. The stability of the calibration standards and extracts were evaluated in the ILV (pp. 26-27; Tables 5-10, pp. 50-55; Tables 14-16, pp. 58-60 of MRID 49807179). The calibration standards and extracts of valifenalate and valifenalate acid were found to be stable up to one week under refrigerated (*ca.* 2°C) conditions; the calibration standards and extracts of p-chlorobenzoic acid were found to be unstable after four weeks under refrigerated (*ca.* 2°C) conditions.
6. The communications between the ECM and ILV were not reported or discussed. The ILV did report that the Study Monitor was contacted regarding the different LOD of valifenalate and valifenalate acid (p. 27 of MRID 49807179). Detailed communication records were not provided. Study personnel listed in the ILV did not include any ECM personnel (p. 6 of MRID 49807179).
7. In the ILV, the time required to complete the extraction of one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 5 hours, followed by *ca.* 2 hours for LC-MS/MS analysis and *ca.* 1 hour for LC-MS/MS evaluation (p. 27 of MRID 49807179). The total time requirement of the method was reported as *ca.* 10 hours in the ILV.

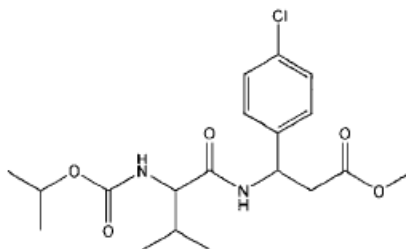


**V. References**

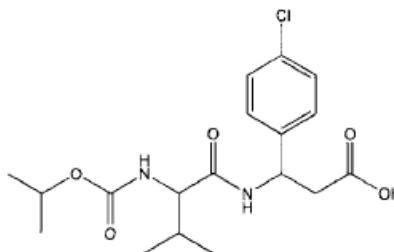
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**DER Attachment 1: Chemical Names and Structures****Valifenalate**

**IUPAC Name:** Not reported  
**CAS Name:** Methyl N-(isopropoxycarbonyl)-L-valyl-(3*R,S*)-2-(4-chlorophenyl)- $\beta$ -alanine  
**CAS Number:** 283159-90-0  
**SMILES String:** Not found

**Valifenalate acid**

**IUPAC Name:** Not reported  
**CAS Name:** (*R,S*)- $\beta$ -alanine, N-((1-methylethoxy)-L-valyl-3-(4-chlorophenyl) acid)  
**CAS Number:** NA  
**SMILES String:** Not found

**p-Chlorobenzoic acid (PCBA)**

**IUPAC Name:** Not reported  
**CAS Name:** 4-Chlorobenzoic acid  
**CAS Number:** 74-11-3  
**SMILES String:** Not found

