

## Analytical method for S-2399 and three of its metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in soil

**Reports:** ECM: EPA MRID No.: 49706427. Bitter, J. 2017. S-2399: Validation of Valent's Method RM-50S, "Determination of Residues of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soil". Valent USA Corporation, USA, Project ID: VP-38934, Report No.: 201700039. Report prepared, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 87 pages. Final report issued February 23, 2017.

ILV: EPA MRID No. 49706090. Rutt, D. 2016. Independent Laboratory Validation for Valent Analytical Method RM-50S "Determination of Residues of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soil". Critical Path Services, USA, Project ID: 15-CPS-001, Report No.: 201600495. Report prepared by Critical Path Services, Garnet Valley, Pennsylvania, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 132 pages. Final report issued August 8, 2016.

**Document No.:** MRIDs 49706427 & 49706090

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, with the exception that the soil characterization was not generated in compliance with GLP at the manufacturer (p. 3 of MRID 49706090). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). An authenticity statement was not provided.

**Classification:** This analytical method is classified as acceptable. However, it could not be determined if the one ILV soil matrix from one terrestrial field dissipation (TFD) study covered the range of soils used in the five TFD studies.


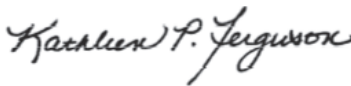
**PC Code:** 090114

**EFED Final Reviewer:** Jessica L. O. Joyce, M.S.,  
Physical Scientist

Signature:  Date: 07/22/2019  
Date: 2019.07.22 14:28:29 -04'00'

**CDM/CSS-Dynamac JV**

**Reviewers:** Lisa Muto, M.S.,  
Environmental Scientist  
Kathleen Ferguson, Ph.D.,  
Environmental Scientist

Signature:  Date: 5/31/18  
Signature:  Date: 5/31/18

*This Data Evaluation Record is a modification of the Tier II DER submitted to the Environmental Fate and Effects Division (EFED) by the registrant. Statistical analyses have been performed according to EFED guidance, and the Executive Summary has been revised. The DER may have been altered by EFED personnel subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## Executive Summary

This analytical method, Valent Method RM-50S, is designed for the quantitative determination of S-2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in soil at the LOQ of 0.01 mg/kg using LC/MS/MS. The LOQ is less than<sup>1</sup> the lowest toxicological level of concern in soil for parent (the degradates have non-definitive endpoints). The ECM validated the method using characterized sandy loam/sandy clay loam soil; the ILV validated the method using characterized loamy sand soil. Although the ILV matrix was from a terrestrial field dissipation study, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the five terrestrial field dissipation studies. The method was validated by the ILV in the first trial with insignificant modifications of the analytical method; however, it was necessary to re-partition the extracts of the LOQ samples of 1'-COOH-S-2840-B due to interferences observed in the extracts of the first partitioning. The ILV report contained a suggestion for the ECM to incorporate solution preparation directions for one solution, but this ILV suggestion did not require the submission of an updated ECM. Based on quantitation results, all ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory.

**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						
S-2399	49706427	49706090	Acceptable	Soil <sup>1,2</sup>	23/02/2017	Valent U.S.A. Corporation	LC/MS/MS	0.01 mg/kg
3'-OH-S-2840								
1'-COOH-S-2840-A								
1'-COOH-S-2840-B								

<sup>1</sup> In the ECM, sandy loam/sandy clay loam soil was the untreated control soil (V-38603-A, 8U-1) obtained from a terrestrial field soil dissipation study in North Dakota (p. 9 of MRID 49706427; p. 16 of MRID 49706464; Northwood, North Dakota; VP-38603). Bulk soil characterization (V-38603-UTC) was performed by Agvise Laboratories, Northwood, North Dakota: 0-30 cm and 30-60 depth – sandy loam, 67% sand 14% silt 19% clay, pH 7.2-8.0 in 1:1 soil:water ratio, 0.79-2.7% organic matter; 60-90 cm depth – sandy clay loam, 65% sand 14% silt 21% clay, pH 8.2 in 1:1 soil:water ratio, 0.52% organic matter (Appendix 2, pp. 75, 85; Appendix 4, pp. 105-107 of MRID 49706464).

<sup>2</sup> In the ILV, loamy sand soil (CPS ID# GS-15-60-1; V-38546-UTC 0-30 cm; 79% sand 16% silt 8% clay, pH 8.1 in 1:1 soil:water ratio, 0.72% organic matter) was supplied by the Sponsor and characterized by Agvise

<sup>1</sup> Lowest toxicological level of concern in 10-day bulk sediment is 5600 µg/kg = 5.6 mg/kg. Also compared to the 10-d OC-normalized sediment: 0.22 g a.i./kg-OC. Assuming %OM from the ILV, and conversion factor of 1.72 to %OC, OC = 2.7/1.72 = 1.6. Therefore, 0.22 g a.i./kg-OC \* 1.6 kg-OC = 0.352 g/kg-soil = 352 mg/kg (MRID 49706489).

Laboratories, Northwood, North Dakota (p. 16; Appendix 1, p. 85 of MRID 49706090; Appendix 4, p. 135 of MRID 49706466). This control soil was from a terrestrial field dissipation (TFD) study in Washington (VP-38546; MRID 49706466).

## I. Principle of the Method

The method determines residues of S-2399 and its metabolites (3'-OH-S-2840, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B) in soil. Soil samples (10.0 g) are weighed into centrifuge tubes (50 mL) and 25 mL acetone/water (4:1, v/v) is added. The samples are shaken for 30 minutes and centrifuged for 5 minutes at 2000 rpm. The extraction step is repeated, combining the extracts in graduated cylinders (100 mL). 25 mL acetone/0.5M HCl (4:1 v/v) is added and the samples shaken for 30 minutes and centrifuged for 5 minutes. The extracts are combined with the first two in the graduated cylinder, 0.5 M sodium acetate solution (2 mL) is added, and the samples mixed. The residues are partitioned into dichloromethane, the solvent evaporated and the residues dissolved in methanol/water (1:1, v/v). Samples are reconstituted in 1:1 (v/v) methanol: water (with or without internal standard) and analysed using high-performance liquid chromatography with tandem mass specific detection ((HPLC/MS-MS) in positive and negative ion modes [MRM (TEM 500°C)] using an Agilent Eclipse XDB-C8 column (150 mm x 4.6 mm, 5 µm particle size; column temperature 40 ± 1°C) and binary gradient elution with mobile phases of A) 5 mM ammonium acetate in HPLC water and B) 5 mM ammonium acetate in methanol [time percent A:B: 0-1.0 min. 65:35, 6.0 min. 10:90, 7.0-10.0 min. 35:65, 11.0-15.0 min. 65:35]. Injection volume 25 µL. Quantification is by internal standards (deuterated analyte species) monitoring the ion transitions in the table below:

Analyte	Ion Mode	Precursor ion Q1 (amu)	Product ion Q3 (amu)
3'-OH-S-2840	-ve	348	175, [130]
3'-OH-S-2840- <i>d3</i>	-ve	351	178
S-2399	+ve	334	<b>258 [238]</b>
S-2399- <i>d3</i>	+ve	337	241, [261]
1'-COOH-S-2840-A	-ve	362	318 [131]
1'-COOH-S-2840-A- <i>d3</i>	-ve	365	321
1'-COOH-S-2840-B	-ve	362	318 [131]
1'-COOH-S-2840-B- <i>d3</i>	-ve	365	321

Values in square parentheses are for qualifier/confirmatory ions

Bolded values were corrected from the Tier II Summary by the reviewer based on p. 13 of MRID 49706427.

Analyte identity was confirmed by comparison of the retention time of the analyte with that of a reference standard using two mass transitions.

The ILV performed the ECM method as written with insignificant modifications of the analytical method (pp. 19-21, 23-24; Table 3, pp. 28-30 of MRID 49706090). The ILV study author recommended that the methanol:water (1:1, v:v) solution preparation be added to the Reagent Solution Preparation section of the ECM. Analyte identification was performed using high-

performance liquid chromatography with tandem mass specific detection (HPLC/MS-MS) in positive and negative ion modes using an Agilent Zorbax Eclipse XDB-C8 column (150 mm x 4.6 mm, 5 µm particle size; column temperature 40 ± 1°C). All other parameters matched those of the ECM. The ILV monitored ion transitions were the same as those of the ECM; however, the quantitation and confirmation ion transitions were inverted for S-2399 (Table 3, p. 30).

The Limit of Quantification (LOQ) for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B residues in soil is 0.01 mg/kg. The Limit of Detection (LOD) is set to be 0.005 mg/kg for each analyte in soil.

## II. Recovery Findings

ECM (MRID 49706427): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of S-2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in a soil matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; p. 14). Only quantitation ion results were reported; a confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data. sandy loam/sandy clay loam soil was the untreated control soil (V-38603-A, 8U-1) obtained from a terrestrial field soil dissipation study in North Dakota (p. 9 of MRID 49706427; p. 16 of MRID 49706464; Northwood, North Dakota; VP-38603). Bulk soil characterization (V-38603-UTC) was performed by Agvise Laboratories, Northwood, North Dakota: 0-30 cm and 30-60 depth – sandy loam, 67% sand 14% silt 19% clay, pH 7.2-8.0 in 1:1 soil:water ratio, 0.79-2.7% organic matter; 60-90 cm depth – sandy clay loam, 65% sand 14% silt 21% clay, pH 8.2 in 1:1 soil:water ratio, 0.52% organic matter (Appendix 2, pp. 75, 85; Appendix 4, pp. 105-107 of MRID 49706464).

ILV (MRID 49706090): Mean recoveries and RSDs were within guidelines for analysis of S-2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in a soil matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; Tables 1-2, pp. 26-27). Only quantitation ion results were reported; a confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data. Loamy sand soil (CPS ID# GS-15-60-1; V-38546-UTC 0-30 cm; 79% sand 16% silt 8% clay, pH 8.1 in 1:1 soil:water ratio, 0.72% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendix 1, p. 85 of MRID 49706090; Appendix 4, p. 135 of MRID 49706466). This control soil was from a terrestrial field dissipation (TFD) study in Washington (VP-38546; MRID 49706466). The method was validated in the first trial with insignificant modifications of the analytical method; however, it was necessary to re-partition the extracts of the LOQ samples of 1'-COOH-S-2840-B due to interferences observed in the extracts of the first partitioning (pp. 14, 19-21, 22-24; Table 3, pp. 28-30 of MRID 49706090). The ILV report contained a suggestion for the ECM to incorporate solution preparation directions for one solution, but this ILV suggestion did not require the submission of an updated ECM.

**Table 2. Initial Validation Method Recoveries for S-2399 and Three of Its Metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Soil<sup>1</sup></b>						
Quantitation Ion Transition						
S-2399	0.010 (LOQ)	5	90.9-99.2	93.3	3.6	3.9
	0.10	5	101.1-106.5	104.2	2.0	1.9
3'-OH-S-2840	0.010 (LOQ)	5	86.5-108.2	95.4	8.4	8.8
	0.10	5	98.8-110.1	104.2	4.2	4.0
1'-COOH-S-2840-A	0.010 (LOQ)	5	71.0-89.1	83.4	7.2	8.6
	0.10	5	77.0-88.4	82.4	4.4	5.3
1'-COOH-S-2840-B	0.010 (LOQ)	7	70.8-93.6	78.5	7.9	10.1
	0.10	5	72.1-77.3	73.8	2.0	2.7
Confirmation Ion Transition						
S-2399	0.010 (LOQ)	5	Recovery data not reported <sup>2</sup>			
	0.10	5				
3'-OH-S-2840	0.010 (LOQ)	5				
	0.10	5				
1'-COOH-S-2840-A	0.010 (LOQ)	5				
	0.10	5				
1'-COOH-S-2840-B	0.010 (LOQ)	7				
	0.10	5				

Data (uncorrected recovery results, pp. Appendix 2, pp. 34-38) obtained from p. 14 of MRID 49706427.

1 The sandy loam/sandy clay loam soil was the untreated control soil (V-38603-A, 8U-1) obtained from a terrestrial field soil dissipation study in North Dakota (p. 9 of MRID 49706427; p. 16 of MRID 49706464; Northwood, North Dakota; VP-38603). Bulk soil characterization (V-38603-UTC) was performed by Agvise Laboratories, Northwood, North Dakota: 0-30 cm and 30-60 depth – sandy loam, 67% sand 14% silt 19% clay, pH 7.2-8.0 in 1:1 soil:water ratio, 0.79-2.7% organic matter; 60-90 cm depth – sandy clay loam, 65% sand 14% silt 21% clay, pH 8.2 in 1:1 soil:water ratio, 0.52% organic matter (Appendix 2, pp. 75, 85; Appendix 4, pp. 105-107 of MRID 49706464).

2 A confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data.

**Table 3: Independent Laboratory Validation for S-2399 and Three of Its Metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Soil<sup>1</sup></b>						
Quantitation Ion Transition						
S-2399	0.010 (LOQ)	5	90.2-102	96.6	4.4	4.6
	0.10	5	94.0-100	97.2	2.5	2.6
3'-OH-S-2840	0.010 (LOQ)	5	88.0-97.6	92.0	4.2	4.6
	0.10	5	90.2-98.6	94.9	3.5	3.7
1'-COOH-S-2840-A	0.010 (LOQ)	5	81.8-89.6	87.1	3.8	4.4
	0.10	5	86.2-90.0	88.0	1.4	1.6
1'-COOH-S-2840-B	0.010 (LOQ) <sup>2</sup>	5	75.2-83.4	77.6	3.3	4.3
	0.10	5	68.0-72.8	71.2	2.0	2.8
Confirmation Ion Transition						
S-2399	0.010 (LOQ)	5	Recovery data not reported <sup>3</sup>			
	0.10	5				
3'-OH-S-2840	0.010 (LOQ)	5				
	0.10	5				
1'-COOH-S-2840-A	0.010 (LOQ)	5				
	0.10	5				
1'-COOH-S-2840-B	0.010 (LOQ)	5				
	0.10	5				

Data (uncorrected recovery results, Appendix 3, pp. 121-123) were obtained from Tables 1-2, pp. 26-27 of MRID 49706090

1 Loamy sand soil (CPS ID# GS-15-60-1; V-38546-UTC 0-30 cm; 79% sand 16% silt 8% clay, pH 8.1 in 1:1 soil:water ratio, 0.72% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendix 1, p. 85 of MRID 49706090; Appendix 4, p. 135 of MRID 49706466). This control soil was from a terrestrial field dissipation (TFD) study in Washington (VP-38546; MRID 49706466).

2 Data from re-partitioned extracts.

3 A confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data.

### III. Method Characteristics

The validated LOQ for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B residues in soil is 0.01 mg/kg (pp. 7, 11; Appendix 2, p. 38 of MRID 49706427; pp. 22, 24 of MRID 49706090). The LOQ was defined by the lowest fortification level validated by the method. The LOD is set to be 0.5 mg/kg for each analyte in soil. In the ECM, the LOD was based on a 10-g sample volume, a 100-mL extract volume, 2.5-mL aliquot volume, 5-mL final volume, a 1x dilution, and a 0.00025 µg/mL calibration standard (as the lowest concentration in the set of calibration standards). In the ILV, both the LOQ and LOD were defined by another ECM of Valent Method RM-50S (Bitter, J., Foster, J. 2015) and unchanged by the ILV.

**Table 4. Method Characteristics**

Analyte		S-2399	3'-OH-S-2840	1'-COOH-S-2840-A	1'-COOH-S-2840-B
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg			
	ILV				
Limit of Detection (LOD)	ECM	0.5 mg/kg			
	ILV				
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	ECM	$r^2 = 0.99976$ (Q)			$r^2 = 0.99976$ (Q - LOQ) <sup>2</sup> $r^2 = 0.99867$ (Q - 10×LOQ)
	ILV	$r^2 = 0.9999$ (Q)		$r^2 = 1.0000$ (Q)	$r^2 = 0.9999$ (Q)
	Conc. range	0.250-10 ng/mL			
Repeatable	ECM <sup>1,3</sup>	Yes at LOQ and 10×LOQ (one characterized soil matrix)			
	ILV <sup>4,5</sup>	Yes at LOQ and 10×LOQ (one characterized soil matrix)			
Reproducible		Yes at LOQ and 10×LOQ			
Specific		Only quantitation ion chromatograms were provided. <sup>6</sup>			
	ECM	Yes, matrix interferences were <13% of the LOQ (based on peak height).	Yes, matrix interferences were <6% of the LOQ (based on peak height).	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.	Yes, matrix interferences were <9% of the LOQ (based on peak area).	Yes, matrix interferences were <3% of the LOQ (based on peak area).	Yes, no matrix interferences were observed (re-partitioned set included).

Data were obtained from p. 14 (recovery results); Appendix 4, pp. 58-62 (calibration curves); Appendix 5, Figures 1-24, pp. 65-87 (chromatograms) of MRID 49706427; Tables 1-2, pp. 26-27 (recovery results); Figures 1-4, pp. 32-35 (correlation coefficients); Figures 5-52, pp. 36-83 (chromatograms) of MRID 49706090; Tier II Summary. Q = Quantitation ion transition.

- 1 Only quantitation ion regression equations and recovery data were reported; a confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data.
- 2 Correlation coefficient ( $r^2$ ) value was reported from the data for the re-partitioned extracts of the LOQ samples.
- 3 In the ECM, sandy loam/sandy clay loam soil was the untreated control soil (V-38603-A, 8U-1) obtained from a terrestrial field soil dissipation study in North Dakota (p. 9 of MRID 49706427; p. 16 of MRID 49706464; Northwood, North Dakota; VP-38603). Bulk soil characterization (V-38603-UTC) was performed by Agvise

- Laboratories, Northwood, North Dakota: 0-30 cm and 30-60 depth – sandy loam, 67% sand 14% silt 19% clay, pH 7.2-8.0 in 1:1 soil:water ratio, 0.79-2.7% organic matter; 60-90 cm depth – sandy clay loam, 65% sand 14% silt 21% clay, pH 8.2 in 1:1 soil:water ratio, 0.52% organic matter (Appendix 2, pp. 75, 85; Appendix 4, pp. 105-107 of MRID 49706464).
- 4 In the ILV, loamy sand soil (CPS ID# GS-15-60-1; V-38546-UTC 0-30 cm; 79% sand 16% silt 8% clay, pH 8.1 in 1:1 soil:water ratio, 0.72% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendix 1, p. 85 of MRID 49706090; Appendix 4, p. 135 of MRID 49706466). This control soil was from a terrestrial field dissipation (TFD) study in Washington (VP-38546; MRID 49706466).
  - 5 The method was validated by the ILV in the first trial with insignificant modifications of the analytical method; however, it was necessary to re-partition the extracts of the LOQ samples of 1'-COOH-S-2840-B due to interferences observed in the extracts of the first partitioning (pp. 14, 19-21, 22-24; Table 3, pp. 28-30 of MRID 49706090). The ILV report contained a suggestion for the ECM to incorporate solution preparation directions for one solution, but this ILV suggestion did not require the submission of an updated ECM.
  - 6 A confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data; therefore, the specificity of the confirmation ion transition does not affect the validity of the method.

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

1. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV loamy sand soil matrix (CPS ID# GS-15-60-1; V-38546-UTC 0-30 cm) was from a terrestrial field dissipation (TFD) study in Washington (VP-38546; MRID 49706466; p. 16; Appendix 1, p. 85 of MRID 49706090; Appendix 4, p. 135 of MRID 49706466). However, five terrestrial field soil dissipation studies were submitted for S-2399 [Washington (VP-38546) – MRID 49706466, Mississippi (VP-38553) – MRID 49706463, California (VP-38586) – MRID 49706467, Ontario, Canada (VP-38593) – MRID 49706465; and North Dakota (VP-38603) – MRID 49706464].
2. The ECM soil characterization was not reported in the study report MRID 49706427; the reviewer obtained the soil characterization data from the corresponding TFD MRID 49706464.
3. The ILV communication with the Sponsor Representative was summarized, but raw communications were not provided for review (p. 23 of MRID 49706090). The decision to re-partition the LOQ samples fortified with 1'-COOH-S-2840-B originated with the ILV study director and the Sponsor Representative (Juliann Bitter; p. 3) from Valent concurred. The reviewer determined that no collusion occurred.
4. In the ECM and ILV, only quantitation ion recovery data and chromatograms were provided. The reviewer noted that a confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data; therefore, the repeatability and specificity of the confirmation ion transition does not affect the validity of the method.
5. The reviewer noted that the ILV appeared to be an independent validation of a previous



ECM of Valent Method RM-50S (Bitter, J., Foster, J. 2015) which was a method only (pp. 22, 24; Appendix 2, Appendix 1, pp. 93-109 of MRID 49706090).

6. The reviewer noted that the Tier II Summary for MRIDs 49706077 & 49706451 contained a table (Table IIA 4.4/04) which contained data from MRIDs 49706451 (IIA 4.4/04), 49706427 (IIA 4.4/01) & 49706090 (IIA 4.4/02).

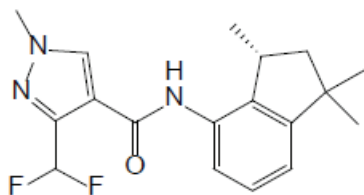
The reviewer also noted that the ECM monitored ion transition table in the Tier II Summary erroneously reported that the monitored ion transitions for S-2399 were  $m/z$  334  $\rightarrow$  238 (Q) and  $m/z$  334  $\rightarrow$  258 (C), when ECM MRID 49706427 reported that the monitored ion transitions for S-2399 were  $m/z$  334  $\rightarrow$  258 (Q) and  $m/z$  334  $\rightarrow$  238 (C; p. 13 of MRID 49706427)

7. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7, 11; Appendix 2, p. 38 of MRID 49706427; pp. 22, 24 of MRID 49706090). The LOQ was defined by the lowest fortification level validated by the method. In the ECM, the LOD was based on a 10-g sample volume, a 100-mL extract volume, 2.5-mL aliquot volume, 5-mL final volume, a 1x dilution, and a 0.00025  $\mu\text{g/mL}$  calibration standard (as the lowest concentration in the set of calibration standards). In the ILV, both the LOQ and LOD were defined by another ECM of Valent Method RM-50S (Bitter, J., Foster, J. 2015) and unchanged by the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
8. The reviewer noted that the experiment start and termination dates were April 28, 2015 and August 12, 2015, respectively (p. 5 of MRID 49706427).
9. It was reported for the ILV that one sample set of 13 samples required *ca.* 8 hours to extract (p. 23 of MRID 49706090). LC/MS/MS analysis required *ca.* 6.5 hours, Therefore, extraction of the 13 samples and analysis of the results required *ca.* 1.5 days total.

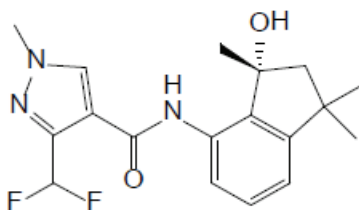
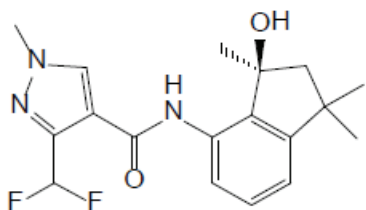
## V. References

- 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.

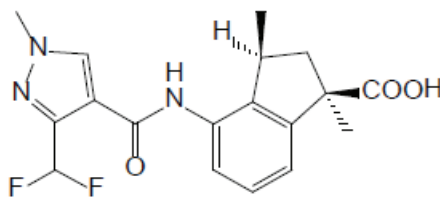
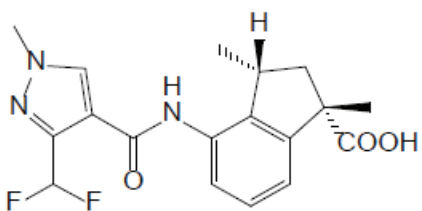
## DER Attachment 1: Chemical Names and Structures



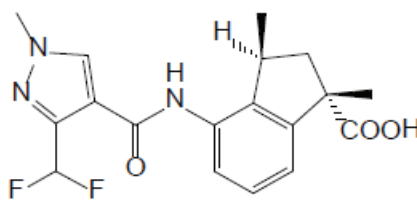
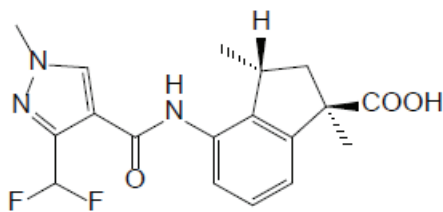
S-2399 (MW = 333.4)

3-(Difluoromethyl)-1-methyl-*N*-[(3'*R*)-1',1',3'-trimethyl-2',3'-dihydro-1'*H*-inden-4'-yl]-1*H*-pyrazole-4-carboxamide

3'-OH-S-2840 (MW = 349.4)

3-(Difluoromethyl)-*N*-[3'-hydroxy-(3'*R*)-1',1',3'-trimethyl-2',3'-dihydro-1'*H*-inden-4'-yl]-1-methyl-1*H*-pyrazole-4-carboxamide3-(Difluoromethyl)-*N*-[3'-hydroxy-(3'*S*)-1',1',3'-trimethyl-2',3'-dihydro-1'*H*-inden-4'-yl]-1-methyl-1*H*-pyrazole-4-carboxamide

1'-COOH-S-2840-A (MW = 363.4)

4'-([3-(Difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl]carbonyl)amino-(1'*R*,3'*S*)-1',3'-dimethyl-2',3' dihydro-1'*H*-indene-1'-carboxylic acid4'-([3-(Difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl]carbonyl)amino-(1'*S*,3'*R*)-1',3'-dimethyl-2',3' dihydro-1'*H*-indene-1'-carboxylic acid

1'-COOH-S-2840-B (MW = 363.4)

4'-([3-(Difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl]carbonyl)amino-(1'*R*,3'*R*)-1',3'-dimethyl-2',3' dihydro-1'*H*-indene-1'-carboxylic acid4'-([3-(Difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl]carbonyl)amino-(1'*S*,3'*S*)-1',3'-dimethyl-2',3' dihydro-1'*H*-indene-1'-carboxylic acid

