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 sediment and soil

Reports: ECM: EPA MRID No.: 50711302. Foster, J. 2016. S-2399: Validation of Valent Method RM-50S-1, "Determination of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Sediment and Soil" (Amendment #1). Valent Technical Center, USA, Project ID: VP-39207. Report prepared, sponsored and submitted by Valent Technical Center, Valent U.S.A. LLC, Dublin, California; 215 pages. Final report issued May 19, 2016, and Amended report issued August 16, 2019.

ILV: EPA MRID No. 49706451. Moate, T. 2017. S-2399: Independent Laboratory Validation for Valent U.S.A Corporation’s Residue Analytical Method for the Determination of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Sediment and Soil (Method Number: RM-50S-1). Golden Pacific Laboratories, USA, Project ID: 160687, Report No: 201700096. Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 166 pages. Final report issued March 27, 2017.

Document No.: MRIDs 50711302 & 49706451

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, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not provided. A Report Signatures page was provided (p. 5). The Report Amendment was provided in Appendix 6 (Appendix 6, pp. 214-215).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, with the exception that the sediment and soil characterization was not generated in compliance with GLP at the manufacturer (p. 3 of MRID 49706451). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was provided with the Quality Assurance statement. A Signature and Approvals page was provided (p. 5).

Classification: This analytical method is classified as Acceptable. 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 **Date:** 11/26/2019

Date: 2019.11.26
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CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S., Environmental Scientist Signature: Lisa Muto

Date: 09/30/2019

Mary Samuel, M.S.,
Environmental Scientist

Signature: Mary Samuel

Date: 09/30/2019

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

This analytical method, Valent Method RM-50S-2, 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 sediment and soil at the LOQ of 0.01 mg/kg using LC/MS/MS. The LOQ is less than[1] the lowest toxicological level of concern in sediment and soil for the four analytes. The ECM validated the method using characterized clay sediment; the ILV validated the method using characterized 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 second trial with insignificant modifications of the analytical method and two modifications of the extraction procedure: an added centrifugation and extra sample flask rinsings prior to SPE clean-up. The two ILV modifications of the extraction procedure were necessary for the success of the ILV trial; the first ILV trial failed due to low recoveries, especially at the LOQ. Based on these findings, the original ECM (Valent Method RM-50S-1) was updated by incorporating these modifications. Based on quantitation results, all ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory. The reviewer also noted that, while the method was written for sediments and soils, the internal validation only used a sediment and the ILV only used a soil.

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[1] 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 = 0.36/1.72 = 0.21. Therefore, 0.22 g a.i.kg-OC * 0.21 kg-OC = 0.0462g/kg-soil = 46.2 mg/kg (MRID 49706489).

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 3'-OH-S-2840 1'-COOH-S-2840-A 1'-COOH-S-2840-B	50711302[1]	49706451	Acceptable	Sediment and Soil[2,3]	19/05/2016 (Original) 16/08/2019 (Amendment #1)	Valent U.S.A. Corporation	LC/MS/MS	0.01 mg/kg

1 Re-submission of ECM MRID 49706077 (Valent Analytical Method RM-50S-1) which was amended based on ILV findings to Valent Analytical Method RM-50S-2.

2 In the ECM, the clay sediment was the untreated control sediment (UTC-Sediment) obtained from an aquatic field soil dissipation study in Louisiana (p. 11 of MRID 50711302; Table 1, p. 31 of MRID 49706485; Rapides Parish, Louisiana; VP-38970). Bulk soil characterization results were as follows: USDA soil texture classification – clay; 14% sand, 38% silt, 48% clay; pH 7.1; 1.60% organic matter (UTC-Sediment 0-5 cm; Lab Sample ID# 15-1162; Table 9, p. 39 of MRID 49706485).

3 In the ILV, sand soil (38586-BS-B; V-38586-UTC 0-30 cm; 88% sand, 7% silt, 5% clay; pH 8.5 in 1:1 soil:water ratio; 0.36% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix C, p. 95 of MRID 49706451; Appendix 4, p. 108 of MRID 49706467). This control soil was from a terrestrial field dissipation (TFD) study in California (V-14-38586; MRID 49706467), although it was characterized as loamy sand in the TFD.

I. Principle of the Method

Samples (10.0 ± 0.1 g) of sediment or soil in 50 mL polypropylene centrifuge tubes were fortified, as necessary with 1 µg/mL fortification solutions, then extracted twice with 25 mL acetone:water (4:1, v:v) via shaking on a reciprocating shaker for 30 minutes then centrifuging (*ca*. 5 minutes at *ca*. 4000 rpm; p. 12; Appendix 3, pp. 91, 94, 96-98 of MRID 50711302). Immediately following, the remaining soil pellet was extracted with acetone:0.5M HCl (4:1, v:v) via shaking on a reciprocating shaker for 30 minutes then centrifuging (*ca*. 5 minutes at unreported speed). The combined extract is immediately mixed with 2 mL of 0.5M sodium acetate solution. The extract was adjusted to pH 5 using 1M acetic acid/sodium acetate buffer**.** Based on ILV findings, the ECM was amended to include the statement that the mixture should be centrifuged (*ca*. 5 minutes at *ca*. 4000 rpm) after addition of the 2 mL of 1M acetic acid/sodium acetate buffer if a precipitate forms. The samples were loaded onto an Oasis HLB 20 cc (1 g) SPE cartridge preconditioned with *ca*. 10 mL of methanol and *ca*. 20 mL of HPLC-grade water. All eluent (after sample loading) was collected into a 50-mL polypropylene tube which was emptied into a 100-mL graduated cylinder. The sample flask was rinsed twice with 5-mL methanol rinses, which were then applied to the cartridge. Based on ILV findings, the ECM was amended to include the statement that the sample flask might need to be rinsed with an additional (*ca*. two) 5-mL methanol rinses, which were then applied to the cartridge, to achieve adequate recoveries. Methanol eluents were collected into the 50-mL polypropylene tube which was emptied into a 100-mL graduated cylinder. The 50-mL polypropylene tube was rinsed with *ca*. 5 mL methanol which was added to the extracts. The final volume of the combined extracts was adjusted to 100 mL with methanol. An aliquot (0.5 mL) of the extract was mixed with either 0.5 mL of the 2 µg/L internal standard or 0.5 mL of methanol:water (1:1, v:v) and analyzed via LC/MS/MS. Internal standards were deuterated analytes. The method noted that volumetric flasks should be rinsed with methanol prior to use in sample processing.

Analytes were identified and quantified using an Agilent Technologies 1200 series high-performance liquid chromatography with tandem Applied Biosystems API 4000 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) 10mM ammonium acetate in HPLC water and B) methanol [time percent A:B: 0-1.0 min. 65:35, 6.0 min. 10:90, 7.0-10.0 min. 35:65, 10.5-14.5 min. 10:90, 15.0-19.0 min. 65:35; pp. 12-14; Appendix 3, pp. 94-96 of MRID 50711301]. Injection volume was 25 µL. Deuterated analyte internal standards were monitored. Period 1 of MS/MS identified 1'-COOH-S-2840-A and 1'-COOH-S-2840-B using negative ion mode. Two ion transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 362→318 and *m/z* 362→131 for 1'-COOH-S-2840-A and 1'-COOH-S-2840-B. One ion transition was monitored for the deuterated analyte internal standards: *m/z* 365→321 for 1'-COOH-S-2840-A-d_3 and 1'-COOH-S-2840-B-d_3. Approximate retention times were 5.8 and 6.3 minutes for 1'-COOH-S-2840-A and 1'-COOH-S-2840-B, respectively. Period 2 of MS/MS identified 3'-OH-S-2840 using negative ion mode. Two ion transitions were monitored (quantitation and confirmation, respectively): *m/z* 348→175 and *m/z* 348→130. One ion transition was monitored for the deuterated analyte internal standard: *m/z* 351→178 for 3'-OH-S-2840-d_3. Approximate retention time was 9.0 minutes. Period 3 of MS/MS identified S-2399 using positive ion mode. Two ion transitions were monitored (quantitation and confirmation, respectively): *m/z* 334→238 and *m/z* 334→258. Two ion transitions were monitored for the deuterated analyte internal standard (quantitation and confirmation, respectively): *m/z* 337→241 and *m/z* 337→261 for S-2399-d_3. Approximate retention time was 9.4 minutes. 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 and two modifications of the extraction procedure: the mixture was centrifuged after addition of the 2 mL of 1M acetic acid/sodium acetate buffer (centrifugation was not performed at this point in the ECM) and the Nalgene bottle was rinsed four times total (instead of twice) after transferring the sample to the SPE column (pp. 18-22 of MRID 49706451). The ILV study author noted that the two modifications of the extraction procedure were necessary to achieve acceptable recoveries. 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 Eclipse XDB-C8 column (150 mm x 4.6 mm, 5 µm particle size; column temperature 40°C; Phenomenex Security Guard Cartridge C8 guard column). 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 (Appendix D, pp. 105-106). Approximate retention times were 8.31, 7.95, 5.13, and 5.45 minutes for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B, respectively. The ILV study author noted that the LC/MS/MS period change should be timed to ensure the complete acquisition of 3'-OH-S-2840 and S-2399.

The Limit of Quantification (LOQ) was defined by the lowest fortification level at which acceptable recovery and repeatability data were obtained. The validated LOQ for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B residues in sediment and soil is 0.01

mg/kg (p. 7; Appendix 2, pp. 42, 69, 100 of MRID 50711302; p. 24 of MRID 49706451). The Limit of Detection (LOD) is set to be 0.005 mg/kg for each analyte in sediment and soil.

II. Recovery Findings

ECM (MRID 50711302): 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 sediment matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; p. 15). 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. Clay sediment was the untreated control sediment (UTC-Sediment) obtained from an aquatic field soil dissipation study in Louisiana (p. 11 of MRID 50711302; Table 1, p. 31 of MRID 49706485; Rapides Parish, Louisiana; VP-38970). Bulk soil characterization results were as follows: USDA soil texture classification – clay; 14% sand, 38% silt, 48% clay; pH 7.1; 1.60% organic matter (UTC-Sediment 0-5 cm; Lab Sample ID# 15-1162; Table 9, p. 39 of MRID 49706485).

ILV (MRID 49706451): 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 I-VIII, pp. 29-36). Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Sand soil (38586-BS-B; V-38586-UTC 0-30 cm; 88% sand, 7% silt, 5% clay; pH 8.5 in 1:1 soil:water ratio; 0.36% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix C, p. 95 of MRID 49706451; Appendix 4, p. 108 of MRID 49706467). This control soil was from a terrestrial field dissipation (TFD) study in California (V-14-38586; MRID 49706467). The method was validated in the second trial with insignificant modifications of the analytical method and two modifications of the extraction procedure: the mixture was centrifuged after addition of the 2 mL of 1M acetic acid/sodium acetate buffer (centrifugation was not performed at this point in the ECM) and the Nalgene bottle was rinsed four times total (instead of twice) after transferring the sample to the SPE column (pp. 18-22; 25). The first trial failed due to low recovery all analytes, especially at the LOQ (Tables IX-XVI, pp. 38-45). Since the two modifications of the extraction procedure were necessary for the success of the ILV trial, an updated ECM was submitted incorporating these modifications.

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 Sediment and Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sediment[1]					
	Quantitation Ion Transition					
S-2399	0.010 (LOQ)	5	79.3-89.8	85.1	3.8	4.5
	0.10	5	101.9-107.0	103.7	2.3	2.2
3'-OH-S-2840	0.010 (LOQ)	5	99.2-103.3	100.7	1.6	1.6
	0.10	5	101.2-113.3	106.2	4.7	4.4
1'-COOH-S-2840-A	0.010 (LOQ)	5	83.1-108.9	91.2	10.5	11.6
	0.10	5	85.5-111.8	95.9	9.6	10.1
1'-COOH-S-2840-B	0.010 (LOQ)	5	105.1-116.6	111.5	6.4	5.7
	0.10	5	87.7-101.4	95.4	5.0	5.2
	Confirmation Ion Transition					
S-2399	0.010 (LOQ)	5	Recovery data not reported[2]			
	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, pp. 16-17) obtained from p. 15 of MRID 50711302**.**

[1] The clay sediment was the untreated control sediment (UTC-Sediment) obtained from an aquatic field soil dissipation study in Louisiana (p. 11 of MRID 50711302; Table 1, p. 31 of MRID 49706485; Rapides Parish, Louisiana; VP-38970). Bulk soil characterization results were as follows: USDA soil texture classification – clay; 14% sand, 38% silt, 48% clay; pH 7.1; 1.60% organic matter (UTC-Sediment 0-5 cm; Lab Sample ID# 15-1162; Table 9, p. 39 of MRID 49706485).

[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 Sediment and Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil[1]					
	Quantitation Ion Transition					
S-2399	0.010 (LOQ)	7	80.3-88.0	85.2	2.53	2.97
	0.10	5	80.5-87.6	84.4	2.53	3.00
3'-OH-S-2840	0.010 (LOQ)	7	83.2-89.3	85.5	2.10	2.46
	0.10	5	80.7-88.2	84.3	3.51	4.16
1'-COOH-S-2840-A	0.010 (LOQ)	7	83.1-93.0	87.5	3.72	4.25
	0.10	5	85.7-93.8	89.5	3.42	3.82
1'-COOH-S-2840-B	0.010 (LOQ)	7	81.4-98.1	86.8	5.51	6.35
	0.10	5	86.9-91.6	89.3	1.71	1.91
	Confirmation Ion Transition					
S-2399	0.010 (LOQ)	7	83.8-89.2	86.5	1.70	1.97
	0.10	5	80.1-86.0	83.4	2.32	2.78
3'-OH-S-2840	0.010 (LOQ)	7	84.9-111	99.3	10.2	10.3
	0.10	5	76.6-88.0	83.0	4.70	5.66
1'-COOH-S-2840-A	0.010 (LOQ)	7	83.0-102	94.2	5.78	6.14
	0.10	5	87.8-92.7	90.3	2.05	2.27
1'-COOH-S-2840-B	0.010 (LOQ)	7	83.5-100	90.7	5.88	6.48
	0.10	5	89.5-92.1	90.5	0.965	1.07

Data (uncorrected recovery results, pp. 22-23) were obtained from Tables I-VIII, pp. 29-36 of MRID 49706451

[1] Sand soil (38586-BS-B; V-38586-UTC 0-30 cm; 88% sand, 7% silt, 5% clay; pH 8.5 in 1:1 soil:water ratio; 0.36% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix C, p. 95 of MRID 49706451; Appendix 4, p. 108 of MRID 49706467). This control soil was from a terrestrial field dissipation (TFD) study in California (V-14-38586; MRID 49706467).

III. Method Characteristics

The LOQ was defined by the lowest fortification level at which acceptable recovery and repeatability data were obtained. The validated LOQ for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B residues in sediment and soil is 0.01 mg/kg (p. 7; Appendix 2, pp. 42, 69, 100 of MRID 50711302; p. 24 of MRID 49706451). The LOD is set to be 0.005 mg/kg for each analyte in sediment and soil. In the ECM, the LOD was based on a 10-g sample volume, a 100-mL extract/eluant volume, 0.5-mL aliquot volume, 1-mL final volume, a 1x dilution, and a 0.25 µg/L calibration standard (as the lowest concentration in the set of calibration standards). Both the LOQ and LOD were defined by the ECM and unchanged by the ILV.

Table 7. 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	0.005 mg/kg			
	ILV				
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 0.99908 (Q)	r^2 = 0.99934 (Q)	r^2 = 0.99652 (Q)	r^2 = 0.99906 (Q)
	ILV	r^2 = 0.9996 (Q) r^2 = 0.9998 (C)	r^2 = 0.9996 (Q & C)	r^2 = 0.9972 (Q) r^2 = 0.9988 (C)	r^2 = 0.9998 (Q & C)
	Conc. range	0.250-10 ng/mL			
Repeatable	ECM[1,2]	Yes at LOQ and 10×LOQ (one characterized sediment matrix)			
	ILV[3,4]	Yes at LOQ and 10×LOQ (one characterized soil matrix)			
Reproducible		Yes at LOQ and 10×LOQ			
Specific		Only quantitation ion chromatograms were provided.[5]			
	ECM	Yes, matrix interferences were <20% of the LOQ (based on peak area).	Yes, matrix interferences were <1% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.		Yes, no matrix interferences were observed; minor baseline interference at was noted.	

Data were obtained from p. 7; Appendix 2, pp. 42, 69, 100 (LOQ/LOD); p. 15 (recovery results); Appendix 4, pp. 128, 148, 168, 188 (calibration curves); Appendix 4, Figures 2-80, pp. 129-207 (chromatograms) of MRID 50711302; p. 24 (LOQ/LOD); Tables I-VIII, pp. 29-36 (recovery results); Appendix D, pp. 105-112 (correlation coefficients); Appendix E, Figures 1-48, pp. 113-164 (chromatograms) of MRID 49706451. Q = Quantitation ion transition; C = Confirmation 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 In the ECM, clay sediment was the untreated control sediment (UTC-Sediment) obtained from an aquatic field soil dissipation study in Louisiana (p. 10 of MRID 50711302; Table 1, p. 31 of MRID 49706485; Rapides Parish, Louisiana; VP-38970). Bulk soil characterization results were as follows: USDA soil texture classification – clay; 14% sand, 38% silt, 48% clay; pH 7.1; 1.60% organic matter (UTC-Sediment 0-5 cm; Lab Sample ID# 15-1162; Table 9, p. 39 of MRID 49706485).

3 In the ILV, sand soil (38586-BS-B; V-38586-UTC 0-30 cm; 88% sand, 7% silt, 5% clay; pH 8.5 in 1:1 soil:water ratio; 0.36% organic matter) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix C, p. 95 of MRID 49706451; Appendix 4, p. 108 of MRID 49706467). This control soil was from a terrestrial field dissipation (TFD) study in California (V-14-38586; MRID 49706467).

4 The method was validated by the ILV in the second trial with insignificant modifications of the analytical method and two modifications of the extraction procedure: the mixture was centrifuged after addition of the 2 mL of 1M acetic acid/sodium acetate buffer (centrifugation was not performed at this point in the ECM) and the Nalgene bottle was rinsed four times total (instead of twice) after transferring the sample to the SPE column (pp. 18-22; 25 of MRID 49706451). The first trial failed due to low recovery all analytes, especially at the LOQ (Tables IX-XVI, pp. 38-45). Since the two modifications of the extraction procedure were necessary for the success of the ILV trial, an updated ECM should be submitted incorporating these modifications.

5 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. ECM/ILV method validation set was previously submitted and reviewed for inpyrfluxam and its metabolites in soil and sediment; however, the validation of ECM MRID 49706077 required ILV MRID 49706451 to incorporate two modifications of the extraction procedure: an added centrifugation and extra sample flask rinsings prior to SPE clean-up. The DER for MRIDs 49706077 & 49706451 was written by CDM Smith/CSS JV Primary Reviewer Lisa Muto, with CDM Smith/CSS JV Secondary Reviewer Kathleen Ferguson. The reviewer determined that an updated ECM should be submitted for inpyrfluxam and its metabolites in soil and sediment to include the two ILV modifications of the extraction procedure, since they were necessary for the success of the ILV trial; the first ILV trial failed due to low recoveries, especially at the LOQ (p. 25; Tables IX-XVI, pp. 38-45 of MRID 49706451).

   For this review, ECM data was verified and updated, and ILV data was verified and edited, as needed.

2. MRID 50711302 was an amended report of ECM MRID 49706077 (Valent Analytical Method RM-50S-1; p. 7; Appendix 6, pp. 214-215 of MRID 50711302). In MRID 50711302, the original ECM was amended to Valent Analytical Method RM-50S-2 to include suggestions of the two ILV modifications of the added centrifugation step and extra sample flask rinsings prior to SPE clean-up. Minor modifications for method clarification were also added. No intrinsic method changes occurred with the amendment. The study author noted that the performance data and supporting calibration curves and chromatograms from the original validation of Method RM-50S-1 were not changed; therefore, Method RM-50S-2 was valid since Method RM-50S was valid and no method changes occurred with the amendment.

   MRID 50711302 was also amended to include the original analytical method, RM-50S, and the modified version of the analytical method, RM-50S-2 (Appendix 6, p. 214 of MRID 50711302).

3. 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 soil matrix (sand soil; 38586-BS-B; V-38586-UTC 0-30 cm) was from a terrestrial field dissipation (TFD) study in California (VP-38586; MRID 49706467; p. 18; Appendix C, p. 95 of MRID 49706451; Appendix 4, p. 108 of MRID 49706467). However, five terrestrial field soil dissipation studies were submitted for S-2399 [Washington (V-14-38546) – MRID 49706466, Mississippi (V-14-38553) – MRID 49706463, California (V-14-38586) – MRID 49706467, Ontario, Canada (V-14-38593) – MRID 49706465; and North Dakota (V-14-38603) – MRID 49706464].

   The ECM soil characterization was not reported in the study report MRID 50711302; the reviewer obtained the soil characterization data from the corresponding TFD MRID 49706485.

The reviewer also noted that, while the method was written for sediments and soils, the internal validation only used a sediment and the ILV only used a soil.

4. The ILV communication with the Registrant/Sponsor was provided for review (p. 25; Appendix F, pp. 165-166 of MRID 49706451). The communications involved information exchange and communication of trial status. The Sponsor Representative (Svetlana Bondarenko) from Valent suggested the two ILV extraction procedure modifications; these modifications were implemented and caused the ILV second trial success. However, the reviewer determined that no collusion occurred since Svetlana Bondarenko was not involved in the internal validation of the method, i.e. not listed as an author, approver or reviewer of the Protocol or Final Report of the ECM MRID 50711302.

5. In the ECM and ILV, only quantitation ion 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 specificity of the confirmation ion transition does not affect the validity of the method.

6. 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 (p. 7; Appendix 2, pp. 42, 69, 100 of MRID 50711302; p. 24 of MRID 49706451). The LOQ was defined by the lowest fortification level at which acceptable recovery and repeatability data were obtained. In the ECM, the LOD was based on a 10-g sample volume, a 100-mL extract/eluant volume, 0.5-mL aliquot volume, 1-mL final volume, a 1x dilution, and a 0.25 µg/L calibration standard (as the lowest concentration in the set of calibration standards). Both the LOQ and LOD were defined by the ECM and unchanged by the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

7. It was reported for the ILV that one sample set of 15 samples required 8 hours to extract (p. 21 of MRID 49706451). LC/MS/MS was performed overnight, and data analysis of the results required *ca*. 2 hours the next day.

V. References

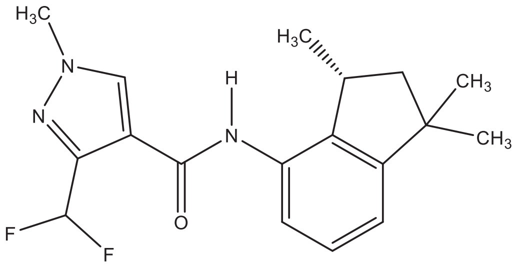
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

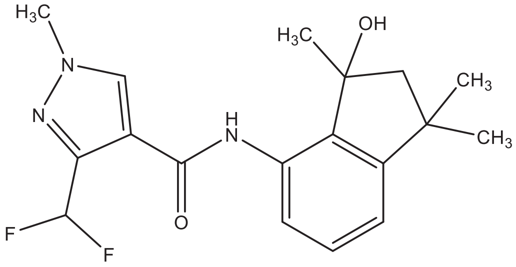
Inpyrfluxam (S-2399)

IUPAC Name:	3-(Difluoromethyl)-N-[(R)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-1H-pyrazole-4-carboxamide
CAS Name:	3-(Difluoromethyl)-N-[(3R)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-1H-pyrazole-4-carboxamide
CAS Number:	1352994-67-2
SMILES String:	O=C(C1=CN(C)N=C1C(F)F)N([H])C2=CC=CC3=C2[C@H](C)CC3(C)C



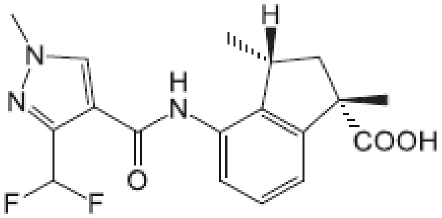
3’-OH-S-2840

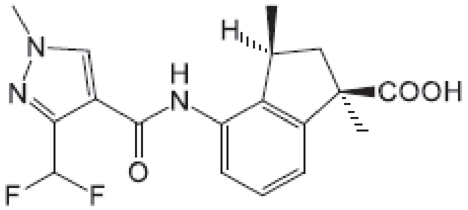
IUPAC Name:	3-(Difluoromethyl)-N-(3-hydroxy-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	O=C(C1=CN(C)N=C1C(F)F)NC2=CC=CC3=C2C(C)(O)CC3(C)C



1’-COOH-S-2840-A

IUPAC Name:	(1S,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid (1R,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	O=C(C1=CN(C)N=C1C(F)F)NC2=CC=CC3=C2[C@@](C)([H])C[C@]3(C)C(O)=O O=C(C1=CN(C)N=C1C(F)F)NC2=CC=CC3=C2[C@](C)([H])C[C@@]3(C)C(O)=O





1’-COOH-S-2840-B

IUPAC Name:	(1R,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid (1S,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	O=C(C1=CN(C)N=C1C(F)F)NC2=CC=CC3=C2[C@@](C)([H])C[C@@]3(C(O)=O)C O=C(C1=CN(C)N=C1C(F)F)NC2=CC=CC3=C2[C@](C)([H])C[C@]3(C(O)=O)C