

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 water

Reports: ECM: EPA MRID No.: 50711301. Foster, J. 2019. S-2399: Validation of Valent Method RM-50W, "Determination of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Surface Water". Amended Report #1. Laboratory Project ID: VP-39100. Report prepared, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 186 pages. Final report issued May 19, 2016; Amended report issued August 14, 2019.

ILV: EPA MRID No. 49706429. Moate, T. 2017. S-2399: Independent Laboratory Validation of 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 Surface Water (Method Number: RM-50W). Golden Pacific Laboratories, LLC (GPL), USA, Project ID: 160698, Report No.: 201700054. Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 155 pages. Final report issued March 8, 2017.

Document No.: MRIDs 50711301 & 49706429

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 of MRID 50711301). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not provided. Report Signatures page was provided (p. 5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, with the exception that the water characterization was not generated in compliance with GLP at the manufacturer (p. 3 of MRID 49706429). 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. Signature and Approvals page was provided (p. 5).


Classification: This analytical method is classified as Acceptable.

PC Code: 090114

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

Signature:  Date: 2019.11.27
Date: 11/27/2019 07:52:45 -05'00'

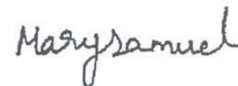
CDM/CSS-Dynamac JV Lisa Muto, M.S.,
Environmental Scientist

Signature:  Date: 09/13/2019

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Signature:



Date:

09/13/2019

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-50W-1, is designed for the quantitative determination of inpyrfluxam (S-2399) and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in water at the LOQ of 1.0 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for the four analytes. The ECM and ILV validated the method using one surface water matrix. The ECM matrix was characterized control source water from a Louisiana S-2399 aquatic field dissipation study while the ILV matrix was characterized commercial bottled spring drinking water. The ILV validated the method in the first trial with insignificant modifications of the analytical method to optimize analyte separation of 3'-OH-S-2840 and S-2399; however, the ILV communications with the Sponsor Representative indicated that the LC column could not be substituted easily. Initially the ILV was using a Phenomenex C8 column which was not working. After the Agilent column was received and incorporated, the ILV chromatography was acceptable. Based on these findings, the original ECM (Valent Method RM-50W) was updated to include a statement that the LC column should not be substituted. Based on quantitation results, all ECM and ILV 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 | 50711301 ¹ | 49706429 | Acceptable | Water ^{2,3} | 19/05/2016 (Original) | Valent U.S.A. Corporation | LC/MS/MS | 1.0 µg/L |
| 3'-OH-S-2840 | | | | | | | | |
| 1'-COOH-S-2840-A | | | | | | | | |
| 1'-COOH-S-2840-B | | | | | 14/08/2019 (Amended) | | | |

1 Re-submission of ECM MRID 49706076 (Valent Analytical Method RM-50W) which was amended based on ILV findings to Valent Analytical Method RM-50W-1. ECM performance data was also included in MRID 50711301.

2 In the ECM, surface water was untreated source water from study VP-38970, entitled "Aquatic Field Dissipation of S-2399 Following Foliar Application of S-2399 2.84 SC to a Flooded Rice Field in Louisiana" (p. 10 of MRID 50711301). The water characterization data was provided on p. 40 of MRID 49706485.

3 In the ILV, surface water was Crystal Geyser Natural Alpine Spring Water by C.G. Roxane in PET bottles bottled in Olancho, California (Appendix C, pp. 83-91 of MRID 49706429). Waters were characterized by the manufacturer for various individual inorganics and organics.

I. Principle of the Method

Samples of surface water (20 mL) were fortified, as necessary with either 1 µg/mL or 0.1 µg/mL fortification solutions, adjusted to *ca.* pH 5 with 1M acetic acid/sodium acetate buffer, and mixed well (pp. 10-12; Appendix 2, pp. 33-35, 38 of MRID 50711301). The samples were loaded onto an Oasis HLB 12 cc (500 mg) SPE cartridge preconditioned with *ca.* 5 mL of methanol and *ca.* 10 mL of HPLC-grade water. The cartridge was washed with 1.7 mL of HPLC-grade water before the analytes were eluted with 9 mL of methanol. The final volume of the samples was adjusted to 10 mL with methanol. An aliquot (0.25 mL) of the extract was mixed with 0.25 mL of HPLC-grade water and 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) 5mM 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, 10.5-12.5 min. 10:90, 13.0-17.0 min. 65:35; pp. 12-14; Appendix 3, pp. 67-69 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. Based on ILV findings, the ECM was amended to include the statement that the LC column (Agilent Eclipse XDB-C8 column) cannot be substituted (pp. 7, 14; Appendix 3, p. 67; Appendix 6, p. 186).

The ILV performed the ECM method as written with insignificant modifications of the analytical method to optimize analyte separation (pp. 19-21 of MRID 49706429). 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. 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. 93-94). Approximate retention times were 8.25, 7.87, 4.99, and 5.30 minutes for S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B, respectively (p. 20). Although the ILV did not make significant modifications to the ECM, the ILV communications with the Sponsor Representative indicated that the LC column could not be substituted easily (p. 21; Appendix F, p. 154). Initially the ILV was using a Phenomenex C8 column which was not working. After the Agilent column was received and incorporated, the ILV chromatography was acceptable. Based on these findings, the ECM was updated to include a statement that the LC column should not be substituted.

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 water is 1.0 µg/L (pp. 7, 17 of MRID 50711301; p. 17 of MRID 49706429). The Limit of Detection (LOD) is set to be 0.5 µg/L for each analyte in water.

II. Recovery Findings

ECM (MRID 50711301): 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 surface water matrix at fortification levels of 1.0 µg/L (LOQ) and 10.0 µg/L (10×LOQ; Summary Tables I-II, p. 8; Appendix 5, pp. 180-183). Only the quantitation ion transition recovery was quantified; a confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to obtain study data. The surface water was untreated source water from study VP-38970, entitled "Aquatic Field Dissipation of S-2399 Following Foliar Application of S-2399 2.84 SC to a Flooded Rice Field in Louisiana" (p. 10). The water characterization data was not provided; the water source was not further described.

ILV (MRID 49706429): Mean recoveries 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 surface water matrix at fortification levels of 1.0 µg/L (LOQ) and 10.0 µg/L (10×LOQ; Tables I-VIII, pp. 27-34). Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable, except for 3'-OH-S-2840 at the LOQ (RSDs were 3.2% and 18.3%). Surface water was Crystal Geyser Natural Alpine Spring Water by C.G. Roxane in PET bottles bottled in Olancho, California (Appendix C, pp. 83-91). Waters were characterized by the manufacturer for various individual inorganics and organics. The method was validated in the first trial with insignificant modifications of the analytical method to optimize analyte separation of 3'-OH-S-2840 and S-2399; however, the ILV communications with the Sponsor Representative indicated that the LC column could not be substituted easily (pp. 19-21, 25; Appendix F, p. 154). Initially the ILV was using a Phenomenex C8 column which was not working. After the Agilent column was received and incorporated, the ILV chromatography was acceptable. Based on these findings, the ECM was updated to include

a statement that the LC column should not be substituted.

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 Water

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|--|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Surface Water¹ | | | | | | |
| Quantitation Ion Transition ² | | | | | | |
| S-2399 | 1.00 (LOQ) | 5 | 97.8-101.0 | 99.3 | 1.3 | 1.3 |
| | 10.0 | 5 | 109.3-112.5 | 111.2 | 1.2 | 1.1 |
| 3'-OH-S-2840 | 1.00 (LOQ) | 5 | 67.5-92.5 | 82.4 | 9.5 | 11.5 |
| | 10.0 | 5 | 78.1-109.7 | 95.8 | 13.4 | 14.0 |
| 1'-COOH-S-2840-A | 1.00 (LOQ) | 5 | 72.1-98.7 | 86.9 | 9.5 | 10.9 |
| | 10.0 | 5 | 67.2-113.8 | 93.7 | 17.4 | 18.6 |
| 1'-COOH-S-2840-B | 1.00 (LOQ) | 5 | 69.9-98.4 | 84.7 | 10.8 | 12.7 |
| | 10.0 | 5 | 71.4-104.0 | 89.5 | 14.6 | 16.3 |

Data (uncorrected recovery results, Appendix 5, pp. 180-183) were obtained from Summary Tables I-II, p. 8; Appendix 5, pp. 180-183 of MRID 50711301.

1 The surface water was untreated source water from study VP-38970, entitled "Aquatic Field Dissipation of S-2399 Following Foliar Application of S-2399 2.84 SC to a Flooded Rice Field in Louisiana" (p. 10). The water source is further described on p. 40 of MRID 49706485.

2 Only the quantitation ion transition recovery was quantified; a confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to obtain study 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 Water

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|----------------------------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Surface Water¹ | | | | | | |
| Quantitation Ion Transition | | | | | | |
| S-2399 | 1.00 (LOQ) | 7 | 81.6-88.6 | 85.3 | 2.90 | 3.40 |
| | 10.0 | 5 | 87.4-93.2 | 89.5 | 2.20 | 2.46 |
| 3'-OH-S-2840 | 1.00 (LOQ) | 7 | 86.9-95.3 | 91.8 | 2.95 | 3.21 |
| | 10.0 | 5 | 86.9-92.9 | 90.7 | 2.90 | 3.20 |
| 1'-COOH-S-2840-A | 1.00 (LOQ) | 7 | 81.0-97.1 | 86.9 | 5.23 | 6.02 |
| | 10.0 | 5 | 83.1-99.0 | 92.4 | 6.20 | 6.71 |
| 1'-COOH-S-2840-B | 1.00 (LOQ) | 7 | 82.8-99.1 | 88.6 | 5.06 | 5.71 |
| | 10.0 | 5 | 86.6-99.1 | 92.4 | 5.68 | 6.15 |
| Confirmation Ion Transition | | | | | | |
| S-2399 | 1.00 (LOQ) | 7 | 81.2-93.6 | 88.3 | 3.72 | 4.21 |
| | 10.0 | 5 | 82.4-86.8 | 85.3 | 1.98 | 2.32 |
| 3'-OH-S-2840 | 1.00 (LOQ) | 7 | 70.7-111 | 86.2 | 15.8 | 18.3 |
| | 10.0 | 5 | 77.4-89.7 | 82.5 | 5.18 | 6.28 |
| 1'-COOH-S-2840-A | 1.00 (LOQ) | 7 | 71.3-90.4 | 83.1 | 6.41 | 7.71 |
| | 10.0 | 5 | 84.4-98.1 | 89.9 | 5.63 | 6.26 |
| 1'-COOH-S-2840-B | 1.00 (LOQ) | 7 | 70.0-95.3 | 80.2 | 9.51 | 11.9 |
| | 10.0 | 5 | 91.5-99.1 | 94.5 | 3.02 | 3.20 |

Data (uncorrected recovery results, pp. 21-22) were obtained from Tables I-VIII, pp. 27-34 of MRID 49706429

¹ Surface water was Crystal Geyser Natural Alpine Spring Water by C.G. Roxane in PET bottles bottled in Olancho, California (Appendix C, pp. 83-91). Waters were characterized by the manufacturer for various individual inorganics and organics.

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 water is 1.0 µg/L (pp. 7, 17; Appendix 3, p. 72 of MRID 50711301; p. 17 of MRID 49706429). The LOD is set to be 0.5 µg/L for each analyte in water. In the ECM, the LOD was calculated based on the product of 10-mL final volume, 4x dilution, and 0.25 µg/L calibration standard (as the lowest concentration in the set of calibration standards) divided by the 20-mL sample volume. Both the LOQ and LOD were defined by the ECM and unchanged by the ILV (p. 24 of MRID 49706429). No justifications, calculations or comparisons to background levels were provided to justify the LOQ.

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 | 1.0 µg/L | | | |
| | ILV | | | | |
| Limit of Detection (LOD) | ECM | 0.5 µg/L | | | |
| | ILV | | | | |
| Linearity (calibration curve r^2 and concentration range) ⁶ | ECM ¹ | $r^2 = 0.99970$ (Q) | $r^2 = 0.99989$ (Q) | $r^2 = 0.99852$ (Q) | $r^2 = 0.99857$ (Q) |
| | | 0.250-10.0 µg/L | | | |
| | ILV | $r^2 = 0.9998$ (Q & C) | $r^2 = 0.9992$ (Q) $r^2 = \mathbf{0.9910}$ (C) ² | $r^2 = 0.9996$ (Q) $r^2 = \mathbf{0.9948}$ (C) ² | $r^2 = 0.9992$ (Q) $r^2 = 0.9994$ (C) |
| 0.250-10.0 ng/mL | | 0.268-10.7 ng/mL | 0.260-10.4 ng/mL | 0.265-10.6 ng/mL | |
| Repeatable | ECM ^{1,3} | Yes at LOQ and 10×LOQ (one uncharacterized water matrix) | | | |
| | ILV ^{4,5} | Yes at LOQ and 10×LOQ (one characterized water matrix) | | | |
| Reproducible | | Yes at LOQ and 10×LOQ | | | |
| Specific | | Only quantitation ion chromatograms were provided. ² | | | |
| | ECM | Yes, matrix interferences were <10% of the LOQ (based on peak area). | Yes, matrix interferences were <6% of the LOQ (based on peak area). | Yes, no matrix interferences were observed; minor baseline interference at was noted. | |
| | ILV | Yes, no matrix interferences were observed. | | Yes, no matrix interferences were observed; minor baseline interference at was noted. | |

Data were obtained from pp. 7, 17; Appendix 3, p. 72 (LOQ/LOD); Summary Tables I-II, p. 8; Appendix 5, pp. 180-183 (recovery data); Appendix 5, pp. 180-183 (calibration coefficients); Appendix 4, pp. 99-178 (calibration curves and representative chromatograms) of MRID 50711301; pp. 17, 24 (LOQ/LOD); Tables I-VIII, pp. 27-34 (recovery data); Appendix D, pp. 93-100 (calibration coefficients); Appendix E, pp. 101-152 (calibration curves and representative chromatograms) of MRID 49706429. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Only the quantitation ion transition recovery was quantified; a confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to obtain study data.

2 A confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data; therefore, the linearity and specificity of the confirmation ion transition does not affect the validity of the method.

3 In the ECM, surface water was untreated source water from study VP-38970, entitled "Aquatic Field Dissipation of S-2399 Following Foliar Application of S-2399 2.84 SC to a Flooded Rice Field in Louisiana" (p. 10 of MRID 50711301). The water characterization data is provided on p. 40 of MRID 49706485.

4 In the ILV, surface water was Crystal Geyser Natural Alpine Spring Water by C.G. Roxane in PET bottles bottled in Olancho, California (Appendix C, pp. 83-91 of MRID 49706429). Waters were characterized by the manufacturer for various individual inorganics and organics.

5 The method was validated by the ILV in the first trial with insignificant modifications of the analytical method to optimize analyte separation of 3'-OH-S-2840 and S-2399; however, the ILV communications with the Sponsor Representative indicated that the LC column could not be substituted easily (pp. 19-21, 25; Appendix F, p. 154 of MRID 49706429). Initially the ILV was using a Phenomenex C8 column which was not working. After the Agilent column was received and incorporated, the ILV chromatography was acceptable. Based on these findings, the ECM was updated to include a statement that the LC column should not be substituted.

6 Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. ECM/ILV method validation set was previously submitted and reviewed for inpyrfluxam and its metabolites in water; however, the ECM MRID 49706076 was a method only without performance data. No water matrix specified or characterized. ILV MRID 49706429 was complete and validated the method as written; however, it was determined that an updated ECM should be submitted to include a statement that the LC column should not be substituted based on ILV communications with the Sponsor Representative (p. 21; Appendix F, p. 154 of MRID 49706429). The DER for MRIDs 49706076 & 49706429 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, complete ECM/ILV method validation set should be submitted for inpyrfluxam and its metabolites in water.

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

2. MRID 50711301 was an amended report of ECM MRID 49706076 (Valent Analytical Method RM-50W; p. 7; Appendix 6, pp. 185-186 of MRID 50711301). In MRID 50711301, the original ECM was amended to Valent Analytical Method RM-50W-1 to include a statement about the LC column based on ILV findings. Minor modifications for method clarification were also added. Method RM-50W-1 contained the statement that it was valid since Method RM-50W was valid and no method changes occurred with the amendment. Additionally, ECM performance data and supporting calibration curves and chromatograms were included in MRID 50711301. The study author noted that the performance data and supporting calibration curves and chromatograms were from the original validation of Method RM-50W.
3. ILV linearity was not satisfactory for the confirmation ion analysis of 3'-OH-S-2840 ($r^2 = 0.9910$) and 1'-COOH-S-2840-B ($r^2 = 0.9948$; Appendix D, pp. 93-100 of MRID 49706429). Linearity is satisfactory when $r^2 \geq 0.995$. 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 linearity of the confirmation ion transition does not affect the validity of the method.
4. 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.
5. 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, 17; Appendix 3, p. 72 of MRID 50711301; p. 17 of MRID 49706429). The LOD is set to be 0.5 $\mu\text{g/L}$ for each analyte in water. In the ECM, the LOD was calculated based on the product of 10-mL final volume, 4x dilution, and 0.25 $\mu\text{g/L}$ calibration standard (as the lowest concentration in the set of calibration standards) divided by the 20-mL sample volume. Both the LOQ

and LOD were defined by the ECM and unchanged by the ILV (p. 24 of MRID 49706429). No justifications, calculations or comparisons to background levels were provided to justify the LOQ. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

6. The ILV communication with the Registrant/Sponsor was provided for review (p. 24; Appendix F, pp. 154-155 of MRID 49706429). The communications involved information exchange and communication of trial status. The reviewer determined that no collusion occurred.
7. It was reported for the ILV that one sample set of 15 samples required 5 hours to extract (p. 21 of MRID 49706429). 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

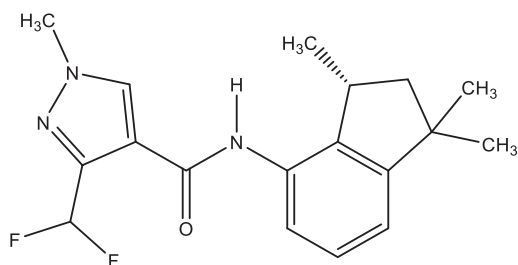
Inpyrfluxam (S-2399)

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



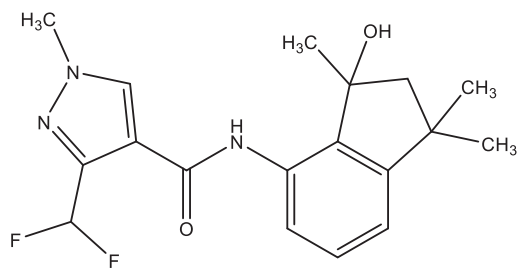
3'-OH-S-2840

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



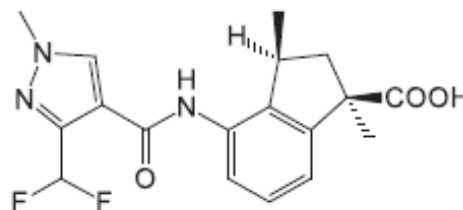
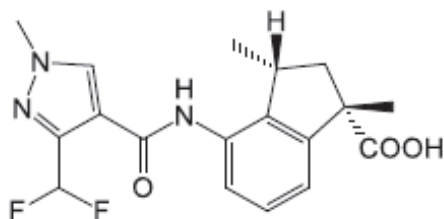
1'-COOH-S-2840-A

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)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

