

Test Material: Sedaxane

MRID: 49805401

Title: SYN548031 and SYN548032 (Metabolites of Sedaxane): SYN548031 and SYN548032 – Residue Method (GRM023.13A) for the Determination of Degradates SYN548031 and SYN548032 in Ground and Surface Water – Analytical Method

MRID: 49805402

Title: SYN548031 and SYN548032 (Metabolites of Sedaxane): Independent Laboratory Validation of “SYN548031 and SYN548032 – Residue Method (GRM023.13A) for the Determination of Degradates SYN548031 and SYN548032 in Ground and Surface Water” – Final ILV Report

EPA PC Code: 129223

OCSPP Guideline: 850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto

Signature: 

Date: 10/7/16

Secondary Reviewer: Kathleen Ferguson

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Date: 10/7/16

Quality Assurance Manager: Joan Gaidos

Signature: 

Date: 10/7/16

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Analytical method for sedaxane metabolites, SYN548031 and SYN548032, in water

Reports: ECM: EPA MRID No.: 49805401. Smith, R. J. 2014. SYN548031 and SYN548032 (Metabolites of Sedaxane): SYN548031 and SYN548032 – Residue Method (GRM023.13A) for the Determination of Degradates SYN548031 and SYN548032 in Ground and Surface Water – Analytical Method. Syngenta Report No. 1781.6992 and Task No. TK0218816. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 155 pages. Final report issued March 3, 2014.

ILV: EPA MRID No. 49805402. Chickering, D. 2014. SYN548031 and SYN548032 (Metabolites of Sedaxane): Independent Laboratory Validation of “SYN548031 and SYN548032 – Residue Method (GRM023.13A) for the Determination of Degradates SYN548031 and SYN548032 in Ground and Surface Water” – Final ILV Report. Syngenta Report/Study No. 81081 and Task No. TK0222971. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 235 pages. Final report issued December 15, 2014.

Document No.: MRIDs 49805401 & 49805402

Guideline: 850.6100


Statements: ECM: No claim of compliance with Good Laboratory Practice (GLP) standards was made; no GLP director participated in this study (p. 3 of MRID 49805401). Signed and dated No Data Confidentiality and GLP were provided (pp. 2-3). A Quality Assurance statement and certification of authenticity was not included.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (p. 3 of MRID 49805402). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 of MRID 49805402). An authenticity statement was not included.

Classification: This analytical method is classified as **ACCEPTABLE**. This classification **does not** reflect consideration of the lowest level of toxicological concern as no toxicity data are available for SYN548031 and SYN548032; however, assuming equal toxicity at the parent the method (LOQ= 0.10 µg/L) is adequate for detecting residues at the lowest toxicological endpoint based on currently available data for parent sedaxane (freshwater fish NOAEC = 110 µg/L). A limited number of samples was noted (n = 3) for all analyses. The LOD was not reported in the ECM and ILV; therefore, it is assumed to be equal to the LOQ.

PC Code: 129223

Reviewer: Rochelle F. H. Bohaty, PhD, Senior Chemist
U.S. EPA


Date: February 24, 2017

Executive Summary

This analytical method, Syngenta Residue Method GRM023.13A, is designed for the quantitative determination of sedaxane metabolites, SYN548031 and SYN548032, in water using LC/MS/MS. The ECM validated the method using groundwater and surface water matrices; the ILV validated the method using surface water with the first trial with minor modifications to the analytical method. Three ion transitions were monitored in the ECM, while only one ion transition was monitored in the ILV. A confirmatory method is not usually required when GC/MS or LC/MS is the primary identification method. In the ECM, the number of samples was insufficient (n = 3) for all analyses. Also, two linear regressions were unsatisfactory for SYN548032, and the purities of the test materials were not reported. The LOD was not reported in the ECM and ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation					
SYN548031	49805401	49805402	Water ^{2,3}	03/03/2014	Syngenta Crop Protection, LLC	LC/MS/MS	0.10 µg/L (0.1 ppb)
SYN548032							

1 SYN548031 = N-[2-(3-cyclopropyl-1,3-dihydroxypropyl)phenyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN548032 = 3-[2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]phenyl]-3-hydroxypropanoic acid.

2 In the ECM, the surface water matrix (pH 5.64, dissolved organic carbon 6.802 mg/L, total hardness 20 mg eq. CaCO₃/L) was collected from the Weweantic River in Wareham, Massachusetts; the ground water matrix (pH 6.70, dissolved organic carbon 0.6854 mg/L, total hardness 76 mg eq. CaCO₃/L) was collected from an on-site well in Wareham, Massachusetts (Table 1, p. 30 of MRID 49805401).

3 In the ILV, the surface water matrix (pH 8.0, total dissolved solids 324 ppm, total hardness 248 mg eq. CaCO₃/L) was collected from a rice paddy and provided by the sponsor (source not further described; ABC Laboratories, Inc. Study No. 80290; pp. 11, 20; Appendix 7, p. 235 of MRID 49805402). The water was fully characterized by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

The method contained the following precautions: 1) high level recoveries and samples with expected residues > 0.1 mg/kg should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL to prevent contamination of the instrument and to minimize possible carry-over issues; and 2) clean gloves should be used when handling controls and when returning to a low concentration from a high concentration (p. 21 of MRID 49805401).

Samples of water (10.0 mL) were transferred to disposable glass vials with PTFE-lined caps (pp. 19-21 of MRID 49805401). After fortification with the mixed fortification solution, as necessary, the samples were diluted into the calibration standard range with acetonitrile or acetonitrile:purified reagent water (20:80, v:v). The sample was transferred analyzed via LC/MS/MS. Samples were stored in the refrigerator in sealed containers when not in use.

Samples were analyzed for SYN548031 and SYN548032 using an Applied Biosystems Sciex API5000 Turbo V Ion Source ESI (500°C; pp. 21-23 of MRID 49805401). The following LC conditions were used: Xbridge C18 column (50 x 2.1 mm, 2.5 µm; column temperature 40.0°C), mobile phase of (A) 0.1% formic acid in purified reagent water and (B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-0.50 min. 95.0:5.0, 3.50-4.00 min. 0.0:100.0, 4.10-5.00 min. 95.0:5.0], and injection volume of 50 µL. The following MS/MS conditions were used: negative ion polarity and multiple reaction monitoring (MRM). Three ion pair transitions were monitored for each analyte (primary, confirmatory 1 and confirmatory 2, respectively): m/z 364.171 → 175.100, m/z 364.171 → 131.200 and m/z 364.171 → 91.200 for SYN548031 and m/z 338.119 → 278.200, m/z 338.119 → 91.200 and m/z 338.119 → 131.100 for SYN548032. Expected retention times were *ca.* 2.4 and 2.1 minutes for SYN548031 and SYN548032, respectively.

In the ILV, the method was performed as written (pp. 14-16; Appendix 4, p. 206 of MIRD 49805402). An AB Sciex API 5000 was used for all analyses. Analytical parameters were the same as those in the ECM, except that only one ion pair was monitored for each analyte: m/z 364 → 175 for SYN548031 and m/z 338 → 278 for SYN548032. Expected retention times were *ca.* 2.6 and 2.3 minutes for SYN548031 and SYN548032, respectively.

The Limit of Quantification (LOQ) for SYN548031 and SYN548032 was reported as 0.10 µg/L (0.10 ppb) in the ECM and the ILV (pp. 16, 26 of MRID 49805401; pp. 8, 19 of MRID 49805402). The Limit of Detection (LOD) was not reported in the ECM and ILV.

II. Recovery Findings

ECM (MRID 49805401): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of sedaxane metabolites SYN548031 and SYN548032 at the LOQ (0.1 $\mu\text{g/L}$), 10 \times LOQ (1.0 $\mu\text{g/L}$), 500 \times LOQ (50.0 $\mu\text{g/L}$) and 50000 \times LOQ (5000 $\mu\text{g/L}$) in two water matrices (Tables 2-7, pp. 31-36; DER Attachment 2). The number of samples was insufficient for all analyses at the LOQ and 10 \times LOQ ($n = 3$). Three ion transitions were monitored for each analyte: primary, confirmatory 1 and confirmatory 2. The surface water matrix (pH 5.64, dissolved organic carbon 6.802 mg/L, total hardness 20 mg eq. CaCO_3/L) was collected from the Weweantic River in Wareham, Massachusetts; the groundwater matrix (pH 6.70, dissolved organic carbon 0.6854 mg/L, total hardness 76 mg eq. CaCO_3/L) was collected from an on-site well in Wareham, Massachusetts (Table 1, p. 30).

ILV (MRID 49805402): Mean recoveries and RSDs were within guideline requirements for analysis of SYN548031 and SYN548032 at the LOQ and 10 \times LOQ in one water matrix (p. 8; Tables 2-3, pp. 24-25). Only one ion pair was monitored for each analyte (primary); a confirmatory method is not usually required when GC/MS or LC/MS is the primary identification method. The surface water matrix (pH 8.0, total dissolved solids 324 ppm, total hardness 248 mg eq. CaCO_3/L) was collected from a rice paddy and provided by the sponsor (source not further described; ABC Laboratories, Inc. Study No. 80290; pp. 11, 20; Appendix 7, p. 235). The water was fully characterized by Agvise Laboratories, Northwood, North Dakota. The method was validated with the first trial with insignificant modifications to the analytical method (pp. 8, 14-19).

Table 2. Initial Validation Method Recoveries for SYN548031 and SYN548032 in Water

Analyte ¹	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ²	Relative Standard Deviation (%)
Surface (River) Water^{3,4}						
Primary Transition						
SYN548031	0.100 (LOQ) ⁵	3	95.1-95.8	95.5	0.4	0.391
	1.00 ⁵	3	94.0-99.0	96.1	2.6	2.70
	50.0	5	98.9-106	101	3.0	2.81
	5000	5	95.0-96.7	95.9	0.8	0.809
SYN548032	0.100 (LOQ)	3	91.9-101	95.3	5.0	5.44
	1.00	3	95.1-110	102	7.5	7.30
	50.0	5	104-111	108	3	2.61
	5000	5	91.0-97.6	94.1	2.7	2.87
Confirmatory transition 1						
SYN548031	0.100 (LOQ) ⁵	3	95.9-98.6	97.3	1.4	1.45
	1.00 ⁵	3	94.1-101	97.1	3.5	3.49
	50.0	5	99.5-106	101	2.8	2.78
	5000	5	94.4-97.7	96.1	1.2	1.23
SYN548032	0.100 (LOQ)	3	83.3-88.9	85.6	2.9	3.39
	1.00	3	84.6-86.0	85.3	0.7	0.814
	50.0	5	101-108	105	3	2.96
	5000	5	89.4-94.5	92.5	2.2	2.36
Confirmatory transition 2						
SYN548031	0.100 (LOQ) ⁵	3	99.0-101	100	1.2	1.24
	1.00 ⁵	3	95.2-105	100	4.9	4.78
	50.0	5	98.8-108	102	3.6	3.31
	5000	5	96.4-98.3	97.2	0.8	0.811
SYN548032	0.100 (LOQ)	3	84.0-103	91.0	10.5	11.7
	1.00	3	87.6-100	94.9	6.4	6.89
	50.0	5	105-111	108	2	2.09
	5000	5	91.8-99.0	94.8	3.7	3.95
Ground (Well) Water^{3,4}						
Primary Transition						
SYN548031	0.100 (LOQ) ⁵	3	95.2-98.4	97.0	1.7	1.71
	1.00 ⁵	3	99.1-105	103	3.0	2.99
	50.0	5	101-105	103	2	1.66
	5000	5	110-116	112	2	2.00
SYN548032	0.100 (LOQ)	3	93.1-104	97.9	5.6	5.67
	1.00	3	106-119	112	7	5.97
	50.0	5	99.2-104	102	1.8	1.71
	5000	5	98.1-109	104	4.6	3.97
Confirmatory transition 1						
SYN548031	0.100 (LOQ) ⁵	3	96.2-98.0	97.0	0.9	0.908
	1.00 ⁵	3	96.1-104	99.8	4.0	4.14
	50.0	5	99.1-104	102	2.2	2.24
	5000	5	105-112	108	3	2.69
SYN548032	0.100 (LOQ)	3	99.4-102	101	1.3	1.38
	1.00	3	103-118	112	8	6.69

Analyte ¹	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ²	Relative Standard Deviation (%)
	50.0	5	98.8-105	102	2.9	2.83
	5000	5	100-108	104	3	3.26
Confirmatory transition 2						
SYN548031	0.100 (LOQ) ⁵	3	101-103	101	1	0.991
	1.00 ⁵	3	102-104	103	1	0.778
	50.0	5	98.5-103	101	1.8	1.86
	5000	5	106-114	109	3	2.79
SYN548032	0.100 (LOQ)	3	95.3-108	100	6.8	6.52
	1.00	3	117-118	117	1	0.451
	50.0	5	99.6-104	102	1.6	1.61
	5000	5	100-106	103	2	1.98

Data (uncorrected recovery results; pp. 24-25) were obtained from Tables 2-7, pp. 31-36 of MRID 49805401 and DER Attachment 2.

1 SYN548031 = N-[2-(3-cyclopropyl-1,3-dihydroxypropyl)phenyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN548032 = 3-[2-[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]phenyl]-3-hydroxypropanoic acid.

2 Standard deviations for the analytes were reviewer-calculated based on data provided in Tables 2-7, pp. 31-36 since the study author did not provide the s.d. values (see DER Attachment 2).

3 The surface water matrix (pH 5.64, dissolved organic carbon 6.802 mg/L, total hardness 20 mg eq. CaCO₃/L) was collected from the Weweantic River in Wareham, Massachusetts; the ground water matrix (pH 6.70, dissolved organic carbon 0.6854 mg/L, total hardness 76 mg eq. CaCO₃/L) was collected from an on-site well in Wareham, Massachusetts (Table 1, p. 30).

4 Three ion pair transitions were monitored for each analyte (primary, confirmatory 1 and confirmatory 2, respectively): m/z 364.171 → 175.100, m/z 364.171 → 131.200 and m/z 364.171 → 91.200 for SYN548031 and m/z 338.119 → 278.200, m/z 338.119 → 91.200 and m/z 338.119 → 131.100 for SYN548032 (p. 23).

5 0.100 µg/L and 1.00 µg/L were nominal fortification rates for SYN548031; actual rates were reported as 0.0995 µg/L and 0.995 µg/L (Tables 2-7, pp. 31-36).

Table 3. Independent Validation Method Recoveries for SYN548031 and SYN548032 in Water

Analyte ¹	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water^{2,3}						
SYN548031	0.10 (LOQ)	5	96-104	99	3.3	3.3
	1.0	5	94-104	98	4.3	4.4
SYN548032	0.10 (LOQ)	5	91-102	95	4.3	4.5
	1.0	5	92-97	94	1.9	2.1

Data (uncorrected recovery results, Table 1, p. 23; Appendix 4, pp. 220-222) were obtained from p. 8; Tables 2-3, pp. 24-25 of MRID 49805402.

1 SYN548031 = N-[2-(3-cyclopropyl-1,3-dihydroxypropyl)phenyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN548032 = 3-[2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]phenyl]-3-hydroxypropanoic acid.

2 The surface water matrix (pH 8.0, total dissolved solids 324 ppm, total hardness 248 mg eq. CaCO₃/L) was collected from a rice paddy and provided by the sponsor (source not further described; ABC Laboratories, Inc. Study No. 80290; pp. 11, 20; Appendix 7, p. 235). The water was fully characterized by Agvise Laboratories, Northwood, North Dakota.

3 Only one ion pair was monitored for each analyte: m/z 364 → 175 for SYN548031 and m/z 338 → 278 for SYN548032 (p. 16).

III. Method Characteristics

The LOQ for SYN548031 and SYN548032, metabolites of sedaxane, was reported as 0.10 µg/L (0.10 ppb) in the ECM and the ILV (pp. 16, 26 of MRID 49805401; pp. 8, 19 of MRID 49805402). In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 80-120% and relative standard deviation of ≤20%. Additionally, the ECM study author stated that the response for an analyte peak at the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time for accurate quantification. No justifications of the LOQ were provided in the ILV. The LOD was not reported in the ECM and ILV.

Table 4. Method Characteristics

Analyte ¹		SYN548031	SYN548032	
Limit of Quantitation (LOQ)		0.10 µg/L		
Limit of Detection (LOD)		Not reported		
Linearity (calibration curve r^2 and concentration range)	ECM (LOQ and 10×LOQ) ²	Ground water	$r^2 = 0.99933$ (primary) $r^2 = 0.99929$ (confirmatory 1) $r^2 = 0.99914$ (confirmatory 2)	$r^2 = 0.99642$ (primary) $r^2 = \mathbf{0.99446}$ (confirmatory 1) $r^2 = 0.99588$ (confirmatory 2)
		Surface water	$r^2 = 0.99864$ (primary) $r^2 = 0.99885$ (confirmatory 1) $r^2 = 0.99853$ (confirmatory 2)	$r^2 = 0.99905$ (primary) $r^2 = 0.99912$ (confirmatory 1) $r^2 = 0.99821$ (confirmatory 2)
		Concentration range	(0.0498-2.49 µg/L)	
	ECM (500×LOQ and 50000×LOQ) ³	Ground water	$r^2 = 0.99989$ (primary) $r^2 = 0.99986$ (confirmatory 1) $r^2 = 0.99852$ (confirmatory 2)	$r^2 = 0.99982$ (primary) $r^2 = 0.99964$ (confirmatory 1) $r^2 = 0.99973$ (confirmatory 2)
		Surface water	$r^2 = 0.99956$ (primary) $r^2 = 0.99912$ (confirmatory 1) $r^2 = 0.99943$ (confirmatory 2)	$r^2 = \mathbf{0.99257}$ (primary) $r^2 = 0.99616$ (confirmatory 1) $r^2 = 0.99542$ (confirmatory 2)
		Concentration range	(0.75-25.0 µg/L)	
	ILV ⁴	$r^2 = 0.9996$ (primary) (0.05-2.50 µg/L)	$r^2 = 0.9998$ (primary) (0.05-2.50 µg/L)	
Repeatable	ECM ⁵	Yes at LOQ and 10×LOQ, but n = 3 . Yes at 500×LOQ and 50000×LOQ		
	ILV ⁶	Yes at LOQ and 10×LOQ.		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes. No matrix interferences were <10% of the LOQ.	Yes. No matrix interferences were <10% of the LOQ; however, baseline noise which interfered with peak integration was noted in surface water chromatograms. ⁷	
		Peak tailing was observed in 500×LOQ and 50000×LOQ chromatograms.		
	ILV	Yes. No matrix interferences were observed.	Yes, no matrix interferences were noted; however, baseline noise was noted near analyte peak. ⁸	

Data were obtained from pp. 16-18, 26; Tables 2-7, pp. 31-36 (recovery results); Figures 27-86, pp. 65-124 (chromatograms); Figures 87-110, pp. 125-148 (calibration curves) of MRID 49805401; pp. 8, 19; Tables 2-3, pp.

24-25 (recovery results); Figures 23-24, pp. 49-50 (calibration curves); Figures 1-8, pp. 27-34 (chromatograms); Appendix 5, pp. 223-226 (raw data) of MRID 49805402; DER Attachment 2.

1 SYN548031 = N-[2-(3-cyclopropyl-1,3-dihydroxypropyl)phenyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN548032 = 3-[2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]phenyl]-3-hydroxypropanoic acid.

2 Three ion pair transitions were monitored for each analyte: primary, confirmatory 1 and confirmatory 2 (p. 23 of MRID 49805401). Calibration standards for fortifications of LOQ and 10×LOQ were prepared in acetonitrile:water matrix, 20:80, v:v (p. 18).

3 Three ion pair transitions were monitored for each analyte: primary, confirmatory 1 and confirmatory 2 (p. 23 of MRID 49805401). Calibration standards for fortifications of 500×LOQ and 50000×LOQ were prepared in water matrix:(acetonitrile:purified reagent water, 20:80, v:v), 10:90, v:v (pp. 17-18).

4 Only one ion pair was monitored for each analyte (p. 16 of MRID 49805402). Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 23-24, pp. 49-50 of MRID 49805402).

5 In the ECM, the surface water matrix (pH 5.64, dissolved organic carbon 6.802 mg/L, total hardness 20 mg eq. CaCO₃/L) was collected from the Weweantic River in Wareham, Massachusetts; the ground water matrix (pH 6.70, dissolved organic carbon 0.6854 mg/L, total hardness 76 mg eq. CaCO₃/L) was collected from an on-site well in Wareham, Massachusetts (Table 1, p. 30 of MRID 49805401).

6 In the ILV, the surface water matrix (pH 8.0, total dissolved solids 324 ppm, total hardness 248 mg eq. CaCO₃/L) was collected from a rice paddy and provided by the sponsor (source not further described; ABC Laboratories, Inc. Study No. 80290; pp. 11, 20; Appendix 7, p. 235 of MRID 49805402). The water was fully characterized by Agvise Laboratories, Northwood, North Dakota.

7 Based on Figures 48-50, pp. 86-88 (LOQ) and Figures 60-62, pp. 98-100 (10×LOQ) of MRID 49805402.

8 Based on Figure 6, p. 32 (LOQ) and Figure 8, p. 34 (10×LOQ) of MRID 49805402.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

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

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM analysis, the number of samples was insufficient (n = 3) for all analyses at the LOQ and 10×LOQ (Tables 2-7, pp. 31-36 of MRID 49805401). OCSPP guidelines recommend that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.
2. ECM linear regressions were unsatisfactory for two SYN548032 calibration curves ($r^2 < 0.995$): $r^2 = 0.99446$ for confirmatory 1 ion transition analysis in groundwater for the LOQ and 10×LOQ analyses and $r^2 = 0.99257$ for primary ion transition analysis in surface water for the 500×LOQ and 50000×LOQ analyses (Figures 87-110, pp. 125-148 of MRID 49805401).
3. The purities of the test materials were not reported in the ECM (Figures 1-2, pp. 39-40 of MRID 49805401).
4. The estimations of the LOQ in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 16, 26 of MRID 49805401; pp. 8, 19 of MRID 49805402). No calculations were reported in ECM or ILV to support the method LOQ. In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 80-120% and relative standard deviation of $\leq 20\%$. Additionally, the ECM study author stated that the response for an analyte peak at the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time for accurate quantification. No

- justifications of the LOQ were provided in the ILV. The LOD was not reported in the ECM and ILV.
5. In ECM and ILV representative LOQ and 10×LOQ chromatograms for SYN548032 in surface water, baseline noise was significant around the analyte peak (Figures 48-50, pp. 86-88; Figures 60-62, pp. 98-100 of MRID 49805401; Figure 6, p. 32; Figure 8, p. 34 of MRID 49805402).
 6. The method calculations allowed for recoveries to be corrected for residues quantified in the controls; however, no residues in the controls were quantified at > 1/3 of the LOQ (pp. 24-25; Figures 27-86, pp. 65-124 of MRID 49805401; Table 1, p. 23; Appendix 4, pp. 220-222 of MRID 49805402).
 7. The reviewer noted a minor typographical error in the ECM and ILV: in the ECM, the SYN548032 confirmatory transition #2 was incorrectly listed as “confirmatory transition #1” in the MRM Conditions table (p. 23 of MRID 49805401); and, in the ILV, the 10×LOQ fortifications were incorrectly listed as “LOQ” in the Fortification Procedures table (p. 17 of MRID 49805402).
 8. The ILV study author provided communication details between the ILV laboratory personnel and the Study Sponsor (p. 18; Appendix 6, pp. 227-234). These included 1) approval of protocol; 2) clarification of method; and 3) update of results of completed trial 1.
 9. It was reported for the ILV that a sample set consisting of approximately 13 samples required *ca.* 4.5 hours for experimental preparation, 6 hours for extraction of samples, 2-3 hours for LC/MS/MS analysis (performed overnight) and 0.5 hour for data manipulation (p. 19 of MRID 49805402).

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.

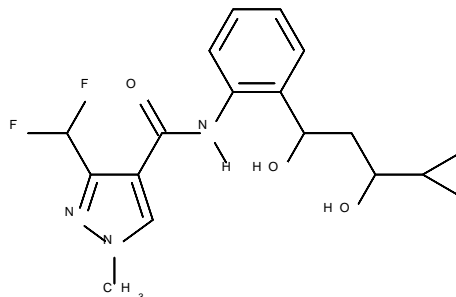
Attachment 1: Chemical Names and Structures**SYN548031 (CSCD668094)**

IUPAC Name: N-[2-(3-cyclopropyl-1,3-dihydroxypropyl)phenyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide

CAS Name: Not reported

CAS Number: Not assigned

SMILES String: Cn1cc(C(=O)Nc2ccccc2C(O)CC(O)C3CC3)c(n1)C(F)F

**SYN548032 (CSCD668095)**

IUPAC Name: 3-[2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]phenyl]-3-hydroxy-propanoic acid

CAS Name: Not reported

CAS Number: Not assigned

SMILES String: Cn1cc(C(=O)Nc2ccccc2C(O)CC(=O)O)c(n1)C(F)F

