

Analytical method for pyrimisulfan and its transformation products, M1, M15 and Imino M18 in water

Reports: ECM: EPA MRID No.: 49683199. Habeeb, S.B. 2016. Method Validation for the Determination of Pyrimisulfan and Metabolites M1, M15 and Imino M18 in Soil, Sediment, and Natural and Surface Water. Report prepared by AgChem Product Development, Ricerca Biosciences LLC, Concord, Ohio, sponsored and submitted by PBI Gordon, Kansas City, Missouri; 258 pages. Ricerca Study No: 032005. Ricerca Document No.: 032005-1. Final report issued May 4, 2016.

ILV: EPA MRID No.: 50106101. Ferguson, L-J. 2016. Independent Laboratory Validation (ILV) Study of Environmental Chemistry Methods for Determination of Pyrimisulfan and Metabolites M1, M15 and Imino M18 in Soil, Sediment, and Natural and Surface Water. Report prepared by AgChem Product Development, Ricerca Biosciences LLC, Concord, Ohio, sponsored and submitted by PBI Gordon, Kansas City, Missouri; 361 pages. Ricerca Study No: 032006. Ricerca Document No.: 032006-1. Final report issued August 9, 2016.

Document No.: MRIDs 49683199 & 50106101

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 49683199). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, which are consistent with the OECD Principles of GLP (p. 3 of MRID 50106101). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **unacceptable**. It could not be determined that the ILV was conducted independently of the ECM. ILV test matrices were the same as those of the ECM. The LODs for the analytes were not reported in the ILV.


PC Code: 079400

EFED Final Reviewer:

Stephen P. Wente, Ph.D.,
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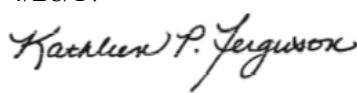
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**CDM/CSS-
Dynamac JV
Reviewers:**

Kathleen Ferguson, Ph.D.,
Environmental Scientist

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Date: 4/26/17

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

Executive Summary

The analytical method, Ricerca Study No. 032005, is designed for the quantitative determination of pyrimisulfan and its transformation products, M1, M15 and Imino M18, in water at the stated LOQ of 2.0 ppb using HPLC/MS/MS. The LOQ is roughly equal to the 7-day IC₅₀ of 2.3 µg a.i./L and greater than the 7-day NOAEC of 1.2 µg a.i./L, which is the lowest toxicological level of concern in water. It could not be determined that ILV MRID 50106101 was conducted independently of ECM MRID 49683199 since both validations were conducted at the same facility (Ricerca Biosciences LLC) and insufficient evidence was provided to support the independence of the two laboratories. Characterized natural and surface water were used for the ILV validation; matrices of the ILV were reported as the same as those of the ECM validation. The ECM method was validated by the ILV with the first trial with insignificant modifications to the LC/MS/MS sample preparation. Analytes were identified using two or three ion transitions. All ILV and ECM data regarding repeatability, accuracy, precision and linearity were satisfactory for all analytes in both matrices. All ILV and ECM data regarding specificity were satisfactory for all analytes in both matrices, except for irregular peak integration of the analyte peak at the baseline in several of the chromatograms. The LODs for the analytes were not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pyrimisulfan	49683199 ²	50106101 ³		Water	04/05/2016	PBI Gordon	LC/MS/MS	2.0 ppb
M1								
M15								
Imino M18								

¹ Pyrimisulfan = (RS)-2'-[(4,6-dimethoxy-pyrimidin-2-yl)(hydroxy)methyl]-1,1-difluoro-6'-(methoxymethyl)methanesulfonanilide; M1 = (RS)-2'-(4-hydroxy-6-methoxy-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; M15 = 2'-(4,6-Dimethoxy-pyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; Imino M18 = 2-[2-(Difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide.

² In the ECM, natural water and surface water were not characterized or described. In the study protocol, the matrices were supposed to be fully characterized by Agvise Laboratories, Northwood, North Dakota, according to GLP (40 CFR Part 160), but this was not reported in the study (Appendix A, pp. 155, 157-158, 160-161 of MRID 49683199).

³ In the ILV, natural water (N 47.33744, W 97.38352; 778 mg equiv. CaCO₃/L, 16.2 ppm total organic carbon; pH not reported) from Golden Lake in Golden Lake, North Dakota, and surface water (N 41.495446, W 80.747337; pH 8.0, 116 mg equiv. CaCO₃/L, 12.7 ppm total organic carbon) from Smokey Oaks Pond in North Bloomfield, Trumbull County, Ohio, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 38; Appendix B, pp. 331-332 of MRID 50106101). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were reported as the same as those of the ECM.

I. Principle of the Method

Water (*ca.* 10 g) in 50-mL polypropylene centrifuge tubes were weighed and fortified with the mixed fortification solution then mixed via hand shaking and a vortex mixer for one minute (pp. 26-27 of MRID 49683199). An aliquot of extract was diluted with water (LOQ: 0.1 mL extract/0.9 mL water; 10×LOQ: 0.1 mL extract/9.9 mL water) into an autosampler vial for analysis by LC/MS/MS.

LC/MS/MS: Samples are analyzed using a MDS-Sciex API 4000 Mass Spectrometer with Shimadzu Nexera UHPLC (LC-30AD pump; pp. 27-29 of MRID 49683199). The following LC conditions were used: Phenomenex Synergi Hydro RP 100 Å column (2.0 mm x 50 mm, 2.5 µ; column temperature ambient), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-1.0 min. 98:2, 4.5 min. 20:80, 4.6-5.6 min. 5:95, 5.7-7.0 min. 98:2], injection volume of 10 µL, and MRM with positive Turbo Spray ionization (Collision Energy 50 V). Two or three ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively, or quantitation, confirmation 1 and confirmation 2, respectively): m/z 420→370 and m/z 420→255 for pyrimisulfan; m/z 406→356 and m/z 406→241 for M1; m/z 418→386, m/z 418→272 and m/z 418→243 for M15; and m/z 324→292, m/z 324→161 and m/z 324→160 for Imino M18. Observed retention time was *ca.* 4.2 minutes for pyrimisulfan, *ca.* 3.4 minutes for M1, *ca.* 4.1 minutes for M15, and *ca.* 2.7 minutes for Imino M18 (Figures 20-21, pp. 73-74; Figures 23-24, pp. 76-77; Figures 44-45, pp. 97-98; Figures 47-48, pp. 100-101; Figures 68-69, pp. 121-122; Figures 71-72, pp. 124-125; Figures 92-93, pp. 145-146; Figures 95-96, pp. 148-149).

The ILV performed the ECM methods for each analyte as written, including analytical methods, except that the dilution factor of most of the pyrimisulfan extracts was modified (decreased) due to poor recoveries which were caused by the non-linearity of the MS response at lower pyrimisulfan concentrations in samples (pp. 42-47, 50-51 of MRID 50106101). Samples were analyzed using a MDS-Sciex API 4000 Mass Spectrometer with Shimadzu LC-30AD (the same equipment as the ECM); column temperature was specified as 30°C. Observed retention times were 3.85 (J) or 4.08 (G) minutes for pyrimisulfan, 3.06 (J) or 3.36 (G) minutes for M1, 3.71 (J) or 3.94 (G) minutes for M15, and 2.29 (J) or 2.48 (G) minutes for Imino M18. Both LC/MS systems J and G were used for ILV identification. A third LC/MS system R was used for Standard Concentration Verification.

In the ECM and ILV, the Limit of Quantification (LOQ) was 2.0 ppb in water for pyrimisulfan, M1, M15, and Imino M18 (pp. 10, 29-30, 33 of MRID 49683199; pp. 7, 47, 50 of MRID 50106101). In the ECM, the Limits of Detection (LOD) were calculated using the standard deviation of the LOQ samples; the calculated LODs for all four analytes ranged 0.16-0.60 ng/g for natural water and 0.15-0.73 ng/g for surface water. In the ILV, the LODs for the analytes were reported from the ECM.

II. Recovery Findings

ECM (MRID 49683199): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyrimisulfan and its transformation products, M1, M15 and Imino M18, at fortification levels of 2.0 ppb (LOQ) and 20 ppb (10 \times LOQ) in water matrices (Tables 9-16, pp. 46-53). Two ion transitions were monitored for pyrimisulfan and M1; three ion transitions were monitored for M15 and Imino M18. Ion transitions were monitored using LC/MS/MS; quantitation and confirmation ion results were fairly comparable, but quantitation ion results generally showed less variation. The natural water and surface water were not characterized or described. In the study protocol, the matrices were supposed to be fully characterized by Agvise Laboratories, Northwood, North Dakota, according to GLP (40 CFR Part 160), but this was not reported in the study (Appendix A, pp. 155, 157-158, 160-161).

ILV (MRID 50106101): Mean recoveries and RSDs were within guidelines for analysis of pyrimisulfan and its transformation products, M1, M15 and Imino M18, at fortification levels of 2.0 ppb (LOQ) and 20 ppb (10 \times LOQ) in water matrices (Tables 1-10, pp. 8-17). Two ion transitions were monitored for pyrimisulfan and M1; three ion transitions were monitored for M15 and Imino M18. Ion transitions were monitored using LC/MS/MS; quantitation and confirmation ion results were fairly comparable, but quantitation ion results generally showed less variation. Natural water (N 47.33744, W 97.38352; 778 mg equiv. CaCO₃/L, 16.2 ppm total organic carbon; pH not reported) from Golden Lake in Golden Lake, North Dakota, and surface water (N 41.495446, W 80.747337; pH 8.0, 116 mg equiv. CaCO₃/L, 12.7 ppm total organic carbon) from Smokey Oaks Pond in North Bloomfield, Trumbull County, Ohio, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 38; Appendix B, pp. 331-332). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were reported as the same as those of the ECM. The method was validated with first trial with insignificant modifications to the LC/MS/MS sample preparation (pp. 42-47, 50-51).

Table 2. Initial Validation Method Recoveries for Pyrimisulfan and Its Transformation Products, M1, M15 and Imino M18, in Water

Analyte ¹	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Natural Water²						
Quantitation Ion ³						
Pyrimisulfan	2.0 (LOQ)	7	84.7-94.8	90.6	4.1	4.5
	20	5	83.5-97.5	90.8	4.9	5.4
M1	2.0 (LOQ)	7	87.3-101.1	94.8	4.6	4.9
	20	5	94.5-105.0	100.0	4.1	4.1
M15	2.0 (LOQ)	7	90.2-99.4	97.0	3.3	3.4
	20	5	94.0-105.0	99.9	5.1	5.1
Imino M18	2.0 (LOQ)	7	99.6-106.7	103.1	2.3	2.2
	20	5	96.0-106.0	101.4	4.5	4.5
Confirmation Ion 1 ³						
Pyrimisulfan	2.0 (LOQ)	7	85.3-105.2	93.8	6.4	6.8
	20	5	80.1-104.5	94.8	9.6	10.2
M1	2.0 (LOQ)	7	83.7-102.9	92.5	7.1	7.7
	20	5	92.5-113.5	101.0	8.1	8.0
M15	2.0 (LOQ)	7	90.2-111.6	103.0	7.7	7.5
	20	5	80.5-100.0	94.6	8.1	8.5
Imino M18	2.0 (LOQ)	7	92.7-110.6	104.4	6.4	6.1
	20	5	89.0-104.1	98.3	6.2	6.3
Confirmation Ion 2 ³						
M15	2.0 (LOQ)	7	70.6-107.3	86.0	11.7	13.6
	20	5	78.1-110.1	91.8	12.2	13.3
Imino M18	2.0 (LOQ)	7	102.1-111.9	108.0	4.1	3.8
	20	5	94.2-101.5	98.8	3.2	3.2
Surface Water²						
Quantitation Ion ³						
Pyrimisulfan	2.0 (LOQ)	7	87.4-96.8	90.4	3.0	3.3
	20	5	92.3-100.4	96.1	3.0	3.1
M1	2.0 (LOQ)	7	91.3-103.7	97.8	4.1	4.2
	20	5	93.3-109.9	102.0	6.6	6.5
M15	2.0 (LOQ)	7	92.9-100.2	95.5	2.5	2.7
	20	5	96.8-104.7	99.7	3.0	3.0
Imino M18	2.0 (LOQ)	7	95.3-106.8	102.1	3.7	3.7
	20	5	95.3-102.4	98.8	2.6	2.6
Confirmation Ion 1 ³						
Pyrimisulfan	2.0 (LOQ)	7	76.0-106.7	91.2	9.6	10.5
	20	5	92.7-107.6	99.0	7.6	7.7
M1	2.0 (LOQ)	7	85.4-110.3	101.0	8.8	8.7
	20	5	84.4-112.1	99.7	10.0	10.1
M15	2.0 (LOQ)	7	83.5-95.8	87.6	4.6	5.3
	20	5	89.2-97.2	92.2	3.8	4.1
Imino M18	2.0 (LOQ)	7	92.5-117.6	100.2	8.7	8.7
	20	5	92.5-105.2	99.9	4.6	4.6
Confirmation Ion 2 ³						
M15	2.0 (LOQ)	7	71.6-97.4	85.0	8.4	9.9

Analyte ¹	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	20	5	72.4-92.5	82.0	7.2	8.8
Imino M18	2.0 (LOQ)	7	91.8-109.4	102.1	6.6	6.5
	20	5	94.4-103.2	100.1	4.1	4.1

Data (uncorrected recovery results; pp. 30-32) were obtained from Tables 9-16, pp. 46-53 of MRID 49683199.

- 1 Pyrimisulfan = (RS)-2'-[(4,6-dimethoxy-pyrimidin-2-yl)(hydroxy)methyl]-1,1-difluoro-6'-(methoxymethyl)methanesulfonamide; M1 = (RS)-2'-(4-hydroxy-6-methoxy-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonamide; M15 = 2'-(4,6-Dimethoxy-pyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonamide; Imino M18 = 2-[2-(Difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide.
- 2 The natural water and surface water were not characterized or described. In the study protocol, the matrices were supposed to be fully characterized by Agvise Laboratories, Northwood, North Dakota, according to GLP (40 CFR Part 160), but this was not reported in the study (Appendix A, pp. 155, 157-158, 160-161).
- 3 Two or three ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively, or quantitation, confirmation 1 and confirmation 2, respectively): m/z 420→370 and m/z 420→255 for pyrimisulfan; m/z 406→356 and m/z 406→241 for M1; m/z 418→386, m/z 418→272 and m/z 418→243 for M15; and m/z 324→292, m/z 324→161 and m/z 324→160 for Imino M18.

Table 3. Independent Validation Method Recoveries for Pyrimisulfan and Its Transformation Products, M1, M15 and Imino M18, in Water

Analyte ¹	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Natural (Lake) Water²						
Quantitation Ion ³						
Pyrimisulfan	2.0 (LOQ)	7	91.4-98.7	94.7	2.5	2.7
	20	5	85.4-96.0	89.4	5.4	6.0
M1	2.0 (LOQ)	7	77.8-95.4	88.5	7.1	8.0
	20	5	90.0-108	95.9	8.3	8.6
M15	2.0 (LOQ)	7	76.4-84.1	81.2	2.4	2.9
	20	5	78.9-86.4	82.5	3.4	4.1
Imino M18	2.0 (LOQ)	7	86.6-108	95.2	6.6	7.0
	20	5	96.5-116	108.9	7.4	6.8
Confirmation Ion 1 ³						
Pyrimisulfan	2.0 (LOQ)	7	91.2-98.5	96.5	3.7	3.8
	20	5	85.0-97.3	90.0	4.9	5.5
M1	2.0 (LOQ)	7	79.1-105	90.6	8.9	9.8
	20	5	77.6-109	94.4	11.4	12.0
M15	2.0 (LOQ)	7	75.9-87.2	81.6	3.8	4.2
	20	5	75.8-100	85.5	10.2	11.9
Imino M18	2.0 (LOQ)	7	70.4-108	93.6	12.2	13.0
	20	5	92.9-121	104.4	13.4	12.9
Confirmation Ion 2 ³						
M15	2.0 (LOQ)	7	75.9-84.9	81.7	3.1	3.7
	20	5	76.1-85.1	80.9	3.9	4.9
Imino M18	2.0 (LOQ)	7	86.6-102	98.2	5.5	5.6
	20	5	96.2-111	105.6	5.7	5.4
Surface (Pond) Water²						
Quantitation Ion ³						
Pyrimisulfan	2.0 (LOQ)	7	83.2-96.4	89.5	3.4	3.8
	20	5	84.5-93.1	88.3	3.7	4.1
M1	2.0 (LOQ)	7	85.3-101	93.2	6.2	6.7
	20	5	92.0-97.7	95.1	2.4	2.6
M15	2.0 (LOQ)	7	85.4-93.4	88.7	3.4	3.8
	20	5	86.7-97.0	91.6	3.9	4.3
Imino M18	2.0 (LOQ)	7	79.0-107	92.1	9.4	10.2
	20	5	95.5-111	103.8	7.7	7.4
Confirmation Ion 1 ³						
Pyrimisulfan	2.0 (LOQ)	7	84.3-98.7	89.6	4.7	5.2
	20	5	87.2-92.6	90.3	2.0	2.2
M1	2.0 (LOQ)	7	87.6-107	94.8	6.6	7.0
	20	5	86.4-92.6	90.7	2.6	2.9
M15	2.0 (LOQ)	7	81.4-102	90.8	6.2	6.8
	20	5	70.1-89.1	83.2	7.6	9.2
Imino M18	2.0 (LOQ)	7	71.0-105	85.2	12.1	14.2
	20	5	90.1-108	97.6	8.7	8.9
Confirmation Ion 2 ³						
M15	2.0 (LOQ)	7	74.2-91.6	83.4	5.7	6.9

Analyte ¹	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	20	5	84.8-103	94.0	7.7	8.2
Imino M18	2.0 (LOQ)	7	79.8-95.1	88.3	4.9	5.6
	20	5	90.6-110	98.7	9.5	9.6

Data (uncorrected recovery results; pp. 48-49) were obtained from Tables 1-10, pp. 8-17 of MRID 50106101.

- 1 Pyrimisulfan = (RS)-2'-[(4,6-dimethoxy-pyrimidin-2-yl)(hydroxy)methyl]-1,1-difluoro-6'-(methoxymethyl)methanesulfonanilide; M1 = (RS)-2'-[(4-hydroxy-6-methoxy-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; M15 = 2'-(4,6-Dimethoxy-pyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; Imino M18 = 2-[2-(Difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide.
- 2 Natural water (N 47.33744, W 97.38352; 778 mg equiv. CaCO₃/L, 16.2 ppm total organic carbon; pH not reported) from Golden Lake in Golden Lake, North Dakota, and surface water (N 41.495446, W 80.747337; pH 8.0, 116 mg equiv. CaCO₃/L, 12.7 ppm total organic carbon) from Smokey Oaks Pond in North Bloomfield, Trumbull County, Ohio, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 38; Appendix B, pp. 331-332). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were reported as the same as those of the ECM.
- 3 Two or three ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively, or quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 420→370 and *m/z* 420→255 for pyrimisulfan; *m/z* 406→356 and *m/z* 406→241 for M1; *m/z* 418→386, *m/z* 418→272 and *m/z* 418→243 for M15; and *m/z* 324→292, *m/z* 324→161 and *m/z* 324→160 for Imino M18.

III. Method Characteristics

In the ECM and ILV, the LOQ was 2.0 ppb in water for pyrimisulfan, M1, M15, and Imino M18 (pp. 10, 29-30, 33 of MRID 49683199; pp. 7, 47, 50 of MRID 50106101). In the ECM, the LOQ was defined as the lowest concurrent fortification that could be successfully processed through the method. The LODs (Method Detection Limit, MDL) were calculated for each monitored ion transition using the standard deviation of the LOQ samples in the following equation:

$$\text{LOD} = t_{0.99} \times S$$

Where *t* = one-tailed *t*-statistic at the 99% confidence level for *n*-1 replicates (where *n* = 7, *t*_{0.99} = 3.143.

S = standard deviation of *n* samples spiked at the LOQ.

The calculated LODs for natural water were 0.19-0.60 ng/g for pyrimisulfan, 0.26-0.55 ng/g for M1, 0.16-0.53 ng/g for M15 and 0.24-0.55 ng/g for Imino M18. The calculated LODs for surface were 0.26-0.40 ng/g for pyrimisulfan, 0.29-0.45 ng/g for M1, 0.21-0.73 ng/g for M15 and 0.15-0.40 ng/g for Imino M18. No method LOD was reported in the ECM. In the ILV, the LOQ and LODs for the analytes were reported from the ECM without further justification.

Table 4. Method Characteristics for Pyrimisulfan and Its Transformation Products, M1, M15 and Imino M18, in Water

Analyte ¹		Pyrimisulfan	M1	M15	Imino M18	
Limit of Quantitation (LOQ)	ECM	2.0 ppb				
	ILV					
Limit of Detection (LOD)	ECM	Natural Water:	0.25 ng (Q) 0.40 ng (C1)	0.29 ng (Q) 0.45 ng (C1)	0.21 ng (Q) 0.48 ng (C1) 0.73 ng (C2)	0.15 ng (Q) 0.40 ng (C1) 0.26 ng (C2)
		Surface Water:	0.19 ng (Q) 0.60 ng (C1)	0.26 ng (Q) 0.55 ng (C1)	0.16 ng (Q) 0.29 ng (C1) 0.53 ng (C2)	0.24 ng (Q) 0.55 ng (C1) 0.42 ng (C2)
	ILV	Not reported				
Linearity (calibration curve r ² and concentration range)	ECM ²	Natural Water:	r ² = 0.9982 (Q)	r ² = 1.0000 (Q)	r ² = 0.9990 (Q)	r ² = 1.0000 (Q)
		Surface Water:	r ² = 0.9984 (Q)	r ² = 0.9998 (Q)	r ² = 0.9990 (Q)	r ² = 1.0000 (Q)
	ILV ³	Natural Water:	r ² = 0.9988 (Q) r ² = 0.9984 (C1)	r ² = 0.9994 (Q) r ² = 0.9992 (C1)	r ² = 0.9980 (Q) r ² = 0.9994 (C1) r ² = 0.9988 (C2)	r ² = 0.9998 (Q) r ² = 0.9988 (C1) r ² = 0.9990 (C2)
		Surface Water:	r ² = 0.9994 (Q) r ² = 0.9998 (C1)	r ² = 0.9996 (Q) r ² = 0.9988 (C1)	r ² = 0.9990 (Q) r ² = 0.9990 (C1) r ² = 0.9976 (C2)	r ² = 0.9994 (Q) r ² = 0.9986 (C1) r ² = 0.9990 (C2)
	Range:		0.10-5.0 ng/mL			
Repeatable	ECM ⁴	Natural Water:	Yes at LOQ and 10×LOQ.			
		Surface Water:				
	ILV ^{5,6}	Natural Water:				
		Surface Water:				
Reproducible		Yes at LOQ and 10×LOQ in natural and surface water matrices.				
Specific	ECM	Only quantitation ion representative chromatograms were provided.				
		Natural Water:	No matrix interferences were observed; however, some peak integration was irregular at the baseline.		No matrix interferences were observed.	
	Surface Water:					
	ILV	Natural Water:	No matrix interferences were observed.	No matrix interferences were observed; however, some C1 peak integration was irregular at the baseline.		No matrix interferences were observed; however, minor baseline noise was noted.
Surface Water:						

Data were obtained from pp. 10, 24, 29-30, 33; Tables 9-16, pp. 46-53 (recovery results); Figures 11-12, pp. 64-65; Figures 35-36, pp. 88-89; Figures 59-60, pp. 112-113; Figures 83-84, pp. 136-137 (calibration curves); Figures 19-24, pp. 72-77; Figures 43-48, pp. 96-101; Figures 67-72, pp. 120-125; Figures 91-96, pp. 144-149 (chromatograms) of MRID 49683199; pp. 7, 47, 50; Tables 1-10, pp. 8-17 (recovery results); Figures 3-4, p. 97; Figures 27-28, p. 119; Figures 51-52, p. 141; Figures 75-76, p. 163; Figures 99-100, p. 185; Figures 123-124, p. 207; Figures 147-148, p. 229; Figures 171-172, p. 251; Figures 195-196, p. 273; Figures 219-220, p. 295 (calibration curves); Figures 19-24, pp. 112-117; Figures 43-48, pp. 134-139; Figures 67-72, pp. 156-161; Figures 91-96, pp. 178-183; Figures 115-120, pp. 200-205; Figures 139-144, pp. 222-227; Figures 163-168, pp. 244-249; Figures 187-192, pp. 266-271; Figures 211-216, pp. 288-293; Figures 235-240, pp. 310-315 (chromatograms) of MRID 50106101. Q = quantitation ion; C1 = confirmation ion 1; C2 = confirmation ion 2. All results reported for Q, C1 and C2 ions unless specified otherwise.

- 1 Pyrimisulfan = (RS)-2'-[(4,6-dimethoxy-pyrimidin-2-yl)(hydroxy)methyl]-1,1-difluoro-6'-(methoxymethyl)methanesulfonanilide; M1 = (RS)-2'-(4-hydroxy-6-methoxy-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; M15 = 2'-(4,6-Dimethoxy-pyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide; Imino M18 = 2-[2-(Difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide.
- 2 In the ECM, correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (pp. 22-24; Figures 11-12, pp. 64-65; Figures 35-36, pp. 88-89; Figures 59-60, pp. 112-113; Figures 83-84, pp. 136-137 of MRID 49683199; DER Attachment 2). Only one set of calibration curves were only provided for each analyte; the reviewer assumed that these were for the quantitation ion.
- 3 In the ILV, correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (pp. 40-41; Figures 3-4, p. 97; Figures 27-28, p. 119; Figures 51-52, p. 141; Figures 75-76, p. 163; Figures 99-100, p. 185; Figures 123-124, p. 207; Figures 147-148, p. 229; Figures 171-172, p. 251; Figures 195-196, p. 273; Figures 219-220, p. 295 of MRID 50106101; DER Attachment 2).
- 4 In the ECM, natural water and surface water were not characterized or described. In the study protocol, the matrices were supposed to be fully characterized by Agvise Laboratories, Northwood, North Dakota, according to GLP (40 CFR Part 160), but this was not reported in the study (Appendix A, pp. 155, 157-158, 160-161 of MRID 49683199).
- 5 In the ILV, natural water (N 47.33744, W 97.38352; 778 mg equiv. CaCO_3/L , 16.2 ppm total organic carbon; pH not reported) from Golden Lake in Golden Lake, North Dakota, and surface water (N 41.495446, W 80.747337; pH 8.0, 116 mg equiv. CaCO_3/L , 12.7 ppm total organic carbon) from Smokey Oaks Pond in North Bloomfield, Trumbull County, Ohio, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 38; Appendix B, pp. 331-332 of MRID 50106101). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were reported as the same as those of the ECM.
- 6 The ILV validated the method with first trial with insignificant modifications to the LC/MS/MS sample preparation (pp. 42-47, 50-51 of MRID 50106101).
- A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined that ILV MRID 50106101 was conducted independently of ECM MRID 49683199 since both validations were conducted at the same facility (Ag Chem Product Development, Ricerca Biosciences LLC, Concord, Ohio) and insufficient evidence was provided to support the independence of the two laboratories (p. 1 of MRID 49683199; p. 1 of MRID 50106101). According to OCSPP guidelines, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. Furthermore, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies. In order to support their independence claim, Ricerca showed that the staff working on each validation were different and reported that no communication occurred between the staff of the initial and independent validations (pp. 17-18 of MRID 49683199; pp. 34-35, 50; Appendix D, p. 340 of MRID 50106101). The ILV reported that communications only took place between the ILV staff and the Sponsor and Study Monitor. However, even though, the ILV made a few insignificant modifications to the LC/MS/MS sample preparation, the equipment lists indicated that both validations used the same API 4000 chromatograph (p. 27 of MRID 49683199; p. 45 of MRID 50106101). The registrant needs to provide additional information to confirm no interactions between staff and no sharing of equipment when both validations occur at the same address.
2. The ILV test matrices were the reported as the same as those of the ECM (Appendix A, pp.

155, 157-158, 160-161 of MRID 49683199; p. 38; Appendix B, pp. 331-332 of MRID 50106101). As well as the matrix similarities supporting the lack of independence of the ILV from the ECM, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

3. In the ILV and ECM, the reviewer noted that the peak integration of the analyte peak was irregular at the baseline in several of the chromatograms, sometimes due to baseline noise (Figures 19-24, pp. 72-77; Figures 43-48, pp. 96-101; Figures 67-72, pp. 120-125; Figures 91-96, pp. 144-149 of MRID 49683199; Figures 19-24, pp. 112-117; Figures 43-48, pp. 134-139; Figures 67-72, pp. 156-161; Figures 91-96, pp. 178-183; Figures 115-120, pp. 200-205; Figures 139-144, pp. 222-227; Figures 163-168, pp. 244-249; Figures 187-192, pp. 266-271; Figures 211-216, pp. 288-293; Figures 235-240, pp. 310-315 of MRID 50106101).
4. In the ECM, only the quantitation ion calibration data and representative chromatograms were provided for review, even though recovery results and calculated LODs were reported for two or three ions for each analyte (Tables 9-16, pp. 46-53; Figures 11-12, pp. 64-65; Figures 35-36, pp. 88-89; Figures 59-60, pp. 112-113; Figures 83-84, pp. 136-137; Figures 19-24, pp. 72-77; Figures 43-48, pp. 96-101; Figures 67-72, pp. 120-125; Figures 91-96, pp. 144-149 of MRID 49683199). However, the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
5. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM (pp. 10, 29-30, 33 of MRID 49683199; pp. 7, 47, 50 of MRID 50106101). In the ECM, the LOQ was defined as the lowest concurrent fortification that could be successfully processed through the method. The LODs (MDL) were calculated using the standard deviation of the LOQ samples in the following equation: $LOD = t_{0.99} \times S$, where t = one-tailed t -statistic at the 99% confidence level for $n-1$ replicates (where $n = 7$, $t_{0.99} = 3.143$) and S = standard deviation of n samples spiked at the LOQ. No method LOD was reported in the ECM. No justification for the LOQ was provided in the ILV. The LODs for the analytes were not reported in the ILV.
6. The communications between the ILV and study developers and sponsors were detailed; communications involved reporting of results and discussion of LC/MS/MS sample dilution modifications (Appendix D, p. 340 of MRID 50106101).
7. The total time required to perform the method for soil, sediment, natural water and surface water (all four sets) was reported as 24 hours for preparation and analysis in the ILV (p. 50 of MRID 50106101).

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

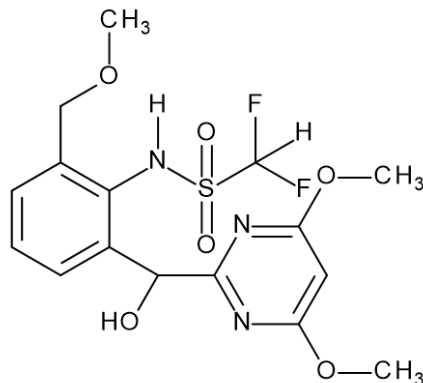
Pyrimisulfan

IUPAC Name: (RS)-2'-[(4,6-dimethoxy-2-pyrimidin-2-yl)(hydroxy)methyl]-1,1-difluoro-6'-methoxymethylmethanesulfonanilide

CAS Name: (RS)-2'-(4,6-dimethoxy-2-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonamide
N-[2-[(4,6-dimethoxy-2-pyrimidin-2-yl)hydroxymethyl]-6-(methoxymethyl)phenyl]-1,1-difluoromethanesulfonamide

CAS Number: 221205-90-9

SMILES String: COCc1cccc(C(O)c2nc(OC)cc(OC)n2)c1NS(=O)(=O)C(F)F

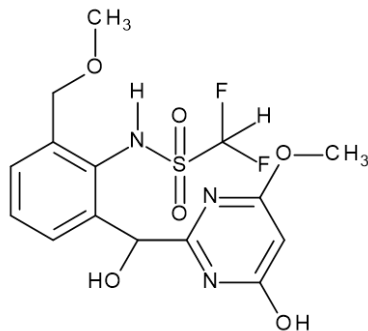
**M1**

IUPAC Name: (RS)-2'-(4-hydroxy-6-methoxy-2-pyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide

CAS Name: Not reported

CAS Number: Not reported

SMILES String: COCc1cccc(C(O)c2nc(O)cc(OC)n2)c1NS(=O)(=O)C(F)F



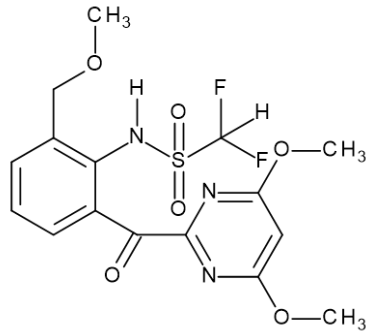
M15

IUPAC Name: 2'-(4,6-Dimethoxypyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide

CAS Name: Not reported

CAS Number: Not reported

SMILES String: COCc1cccc(C(=O)c2nc(OC)cc(OC)n2)c1NS(=O)(=O)C(F)F

**Imino M18 (Imino M18 TFA salt)**

IUPAC Name: 2-[2-(Difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide

CAS Name: Not reported

CAS Number: Not reported

SMILES String: COCc1cccc(C(O)C(=N)N)c1NS(=O)(=O)C(F)F

