

Analytical method for pyraflufen-ethyl and its metabolites E-1, E-2, and E-3 in soil

Reports: ECM: EPA MRID No.: 50336601. Coleman, H. 2017. Method Validation for the Determination of Residues of Pyraflufen-ethyl and Metabolites E-1, E-2 and E-3 in Soil. Study No.: XG/17/002. Report prepared by Battelle UK Ltd., Essex, United Kingdom, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 94 pages. Final report issued March 22, 2017.

ILV: EPA MRID No. 50336602. Watson, G. 2017. Independent Laboratory Validation of analytical method XG/17/002 for the determination of residues of pyraflufen-ethyl and metabolites E-1, E-2 and E-3 in soil by LC-MS/MS. Study Reference No.: RES-00090. Report prepared by ResChem Analytical Limited, Derby, United Kingdom, and sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 117 pages (including page 1a). Final report issued June 23, 2017.

Document No.: MRIDs 50336601 & 50336602

Guideline: 850.6100

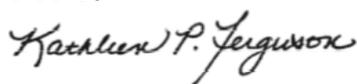
Statements: ECM: The study was conducted in accordance with UK and OECD Good Laboratory Practice (GLP) standards, which are accepted by Regulatory Authorities throughout the European Community, the United States of America and Japan (p. 3; Appendix 14, p. 94 of MRID 50336601). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 14, p. 94). A statement of the authenticity of the study report was included with the quality assurance and GLP statements (pp. 3-4).

ILV: The study was conducted in accordance with OECD and UK GLP standards (p. 2; Appendix C, p. 117 of MRID 50336602). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 1a-2, 4; Appendix C, p. 117). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is classified as supplemental. The communication between the ILV testing facility and the method developer or ECM was not reported. In order for the ECM to potentially fulfill guideline requirements the ILV needs to be supported with documentation of all communications (or lack thereof) that occurred between the ILV laboratory personnel and the ECM personnel. If it can be shown that no disallowed communications occurred, then the study may be accepted to fulfill guideline requirements. The ECM must be written in a way that it is reproducible and can be validated by an independent laboratory without any communication required.

Chromatograms from only selected fortifications were provided. The ECM and ILV soil matrices were the same texture classification with similar matrix characteristics.

PC Code: 030090

EFED Final Reviewer:	Dena Barrett, Chemist	Signature:  Date: 9/18/20
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:  Date: 1/11/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:  Date: 1/11/18

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

This analytical method, Analytical Method XG/17/002, is designed for the quantitative determination of pyraflufen-ethyl and its metabolites E-1, E-2, and E-3 in soil at the LOQ of 0.05 µg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for all analytes. The ECM and ILV used characterized clay and loamy sand soil matrices; matrix characteristics were similar, but not the same. It could not be determined if the ILV was provided with the most difficult and comprehensive matrices with which to validate the method. All analytes were identified using two ion transitions, except for E-1 which was identified using two LC/MS systems. Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method. All submitted ILV and ECM data pertaining to linearity, precision, repeatability, and reproducibility was acceptable. The specificity of the method was determined to be acceptable for all analytes in both matrices in the ECM and ILV, except for ECM and ILV chromatograms of E-3 in clay soil; however, representative chromatograms of 10×LOQ and 100×LOQ fortifications were not provided. The LOD was not discussed in the ILV but was implicitly validated.

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						
Pyraflufen-ethyl	50336601 ¹	50336602 ²		Soil	22/03/2017	Nichino America, Inc.	LC/MS/MS	0.05 µg/kg
E-1								
E-2								
E-3								

¹ In the ECM, clay soil matrix [Lufa Speyer soil type 6S; 22% sand 27% silt 51% clay; pH 7.0 (0.01M CaCl₂), 1.7% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 86% sand 10% silt 4% clay; pH 6.0 (0.01M CaCl₂), 1.6% organic carbon] were used (USDA soil texture classification; p. 18; Appendices 8-9, pp. 87-88 of MRID 50336601). Both soils were obtained from Battelle UK. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, clay soil matrix [Lufa Speyer soil type 6S; $25.6 \pm 3.2\%$ sand $34.4 \pm 2.6\%$ silt $40.0 \pm 2.1\%$ clay; pH 7.1 ± 0.5 (0.01M CaCl₂), $1.73 \pm 0.05\%$ organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; $75.8 \pm 3.9\%$ sand $16.3 \pm 2.5\%$ silt $7.9 \pm 1.8\%$ clay; pH 5.5 ± 0.1 (0.01M CaCl₂), $1.61 \pm 0.15\%$ organic carbon] were used (USDA soil texture classification; p. 13; Appendix B, pp. 113-115 of MRID 50336602). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Soil samples (50 g) were fortified with fortification solutions of pyraflufen-ethyl, E-1, E-2, and E-3 in a 25-mL polypropylene tube (pp. 19-22; Appendices 1-3, pp. 80-82 of MRID 50336601). The samples were sequentially extracted twice with acetonitrile:1 M ammonium chloride solution (4:1, v:v; 1 x 30 mL and 1 x 15 mL) and once with acetonitrile:1 M hydrochloride acid solution (4:1, v:v; 1 x 15 mL) via shaking on a mechanical shaker for 15 minutes. After centrifugation (4000 rpm for 2 minutes), the supernatant transferred to a clean 70-mL vessel. The volume of the combined extract was adjusted to 70 mL using acetonitrile:1 M hydrochloride acid solution (4:1, v:v). After centrifugation (4000 rpm for 2 minutes), an aliquot of the sample was transferred to an autosampler vial and analyzed by HPLC/MS/MS.

Samples were analyzed for pyraflufen-ethyl, E-1, E-2, and E-3 using an Agilent 1290 HPLC coupled to a MDS Sciex API 5500 MS equipped with a Zorbax SB-C3 column (4.6 mm x 150 mm, 5.0 μ m; column temperature 50°C) using a mobile phase of (A) 0.2% acetic acid in water and (B) 0.2% acetic acid in acetonitrile [percent A:B at 0.0 min. 90:10, 10.0-11.0 min. 5:95, 11.1-11.5 min. 90:10] with MS/MS-ESI (electrospray ionization) detection in positive ion mode (pyraflufen-ethyl, E-2, and E-3) or negative ion mode (E-1) and multiple reaction monitoring (MRM; pp. 22-24; Appendix 2, pp. 81-82 of MRID 50336601). Injection volume was 100 μ L. Analytes, except E-1, were identified using two ion transitions (quantitation and confirmation, respectively): m/z 413 \rightarrow 339 and m/z 413 \rightarrow 289 for pyraflufen-ethyl, m/z 327 \rightarrow 277 and m/z 329 \rightarrow 279 for E-2, and m/z 341 \rightarrow 291 and m/z 341 \rightarrow 276 for E-3. For E-1, only one ion transition was monitored with this LC/MS/MS system: m/z 383 \rightarrow 274, the quantitation ion transition. Expected retention times were *ca.* 9.9, 8.9, 8.8, and 9.8 minutes for pyraflufen-ethyl, E-1, E-2, and E-3, respectively.

For the confirmation analysis of E-1, samples were analyzed using the same LC/MS/MS instruments equipped with a Luna Phenyl Hexyl column (4.6 mm x 150 mm, 5.0 μ m; column temperature 50°C) using a mobile phase of (A) 0.2% acetic acid in water and (B) 0.2% acetic acid in acetonitrile [percent A:B at 0.0 min. 90:10, 3.0-5.0 min. 5:95, 5.1-5.5 min. 90:10] with MS/MS-ESI (electrospray ionization) detection in negative ion mode and multiple reaction monitoring (MRM; pp. 22-24; Appendix 2, pp. 81-82 of MRID 50336601). Injection volume was 100 μ L. The same ion transition was monitored for confirmation of E-1: m/z 383 \rightarrow 274. Expected retention time was *ca.* 4.6 minutes for E-1.

In the ILV, the ECM was performed as written with insignificant modifications to the analytical parameters (pp. 16-18, 20 of MRID 50336602). An Agilent 1290 Series HPLC coupled to an AB Sciex API 5500 MS was used for analyte identification. The injection volume was 50 μ L for all analyses. Pyraflufen-ethyl, E-1, E-2, and E-3 were identified using the same ion transitions. For the Zorbax SB-C3 column, expected retention times were *ca.* 9.1, 6.7, 8.2, and 9.0 minutes for pyraflufen-ethyl, E-1, E-2, and E-3, respectively. For the Luna Phenyl Hexyl column, expected retention time was *ca.* 4.4 minutes for E-1. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for soil was 0.05 μ g/kg in the ECM and ILV (pp. 15, 29-30 of MRID 50336601; pp. 10, 14-15, 42 of MRID 50336602). The Limit of Detection (LOD) was reported as 0.01 μ g/kg in the ECM. In the ILV, the LOD was confirmed to be less than 30% of

the LOQ, as demonstrated by the lowest mixed calibration standard (0.01 ng/mL; equivalent to 28% of the LOQ).

II. Recovery Findings

ECM (MRID 50336601): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of pyraflufen-ethyl, E-1, E-2, and E-3 at fortification levels of 0.05 $\mu\text{g}/\text{kg}$ (LOQ), 0.5 $\mu\text{g}/\text{kg}$ (10 \times LOQ), and 5.0 $\mu\text{g}/\text{kg}$ (100 \times LOQ) in two soil matrices (pp. 13-15; Tables 7-14, pp. 36-43). Pyraflufen-ethyl, E-2, and E-3 were identified using two ion transitions; E-1 was identified using two LC/MS/MS conditions. Performance data (recovery results) from quantitation and confirmation analyses were comparable. Clay soil matrix [Lufa Speyer soil type 6S; 22% sand 27% silt 51% clay; pH 7.0 (0.01M CaCl₂), 1.7% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 86% sand 10% silt 4% clay; pH 6.0 (0.01M CaCl₂), 1.6% organic carbon] were used (USDA soil texture classification; p. 18; Appendices 8-9, pp. 87-88). Both soils were obtained from Battelle UK. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50336602): Mean recoveries and RSDs were within guideline requirements for analysis of pyraflufen-ethyl, E-1, E-2, and E-3 at fortification levels of 0.05 $\mu\text{g}/\text{kg}$ (LOQ), 0.5 $\mu\text{g}/\text{kg}$ (10 \times LOQ), and 5.0 $\mu\text{g}/\text{kg}$ (100 \times LOQ) in two soil matrices (Tables 17-32, pp. 26-41). Pyraflufen-ethyl, E-2, and E-3 were identified using two ion transitions; E-1 was identified using two LC/MS/MS conditions. Performance data (recovery results) from quantitation and confirmation analyses were comparable. Clay soil matrix [Lufa Speyer soil type 6S; 25.6 \pm 3.2% sand 34.4 \pm 2.6% silt 40.0 \pm 2.1% clay; pH 7.1 \pm 0.5 (0.01M CaCl₂), 1.73 \pm 0.05% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 \pm 3.9% sand 16.3 \pm 2.5% silt 7.9 \pm 1.8% clay; pH 5.5 \pm 0.1 (0.01M CaCl₂), 1.61 \pm 0.15% organic carbon] were used (USDA soil texture classification; p. 13; Appendix B, pp. 113-115). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method (pp. 10, 16-18, 20, 47).

Table 2. Initial Validation Method Recoveries for Pyraflufen-ethyl and its Metabolites E-1, E-2, and E-3 in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Soil						
Quantitation Ion Transition						
Pyraflufen-ethyl	0.05	6	91.3-99.7	94.8	3.5	3.6
	0.5	6	82.9-90.2	85.0	3.0	3.5
	5.0	6	82.6-91.0	85.4	3.0	3.5
E-1	0.05	6	88.5-106	94.1	6.5	6.9
	0.5	6	65.3-78.1	73.0	4.3	5.9
	5.0	6	69.5-82.9	74.5	4.5	6.1
E-2	0.05	6	88.8-110	95.3	7.7	8.1
	0.5	6	83.7-94.1	87.7	3.8	4.3
	5.0	6	84.9-90.7	86.9	2.1	2.4
E-3	0.05	6	90.7-112	99.9	8.6	8.6
	0.5	6	82.6-92.4	86.6	3.5	4.1
	5.0	6	81.5-89.9	85.4	3.5	4.0
Confirmation Ion Transition (Confirmation Method)						
Pyraflufen-ethyl	0.05	6	86.5-111	96.3	11.1	11.5
	0.5	6	81.8-89.3	85.8	2.6	3.0
	5.0	6	81.5-94.9	85.2	5.0	5.8
E-1	0.05	6	71.7-83.2	74.2	4.5	6.1
	0.5	6	63.9-85.4	77.4	8.3	10.7
	5.0	6	74.5-84.0	77.4	3.8	4.9
E-2	0.05	6	82.3-115	91.3	11.8	13.0
	0.5	6	82.9-89.9	86.9	2.6	3.0
	5.0	6	83.2-91.3	88.2	3.0	3.4
E-3	0.05	6	101-117	107	5.6	5.2
	0.5	6	81.5-91.6	87.5	3.3	3.8
	5.0	6	80.9-93.0	85.0	4.6	5.4
Loamy Sand Soil						
Quantitation Ion Transition						
Pyraflufen-ethyl	0.05	6	68.9-82.1	76.9	5.4	7.0
	0.5	6	62.2-99.5	74.5	14.8	19.8
	5.0	5	99.2-108	103	3.5	3.4
E-1	0.05	6	76.8-100	90.6	8.4	9.3
	0.5	6	59.7-93.6	70.5	12.1	17.2
	5.0	5	89.4-92.8	91.2	1.2	1.3
E-2	0.05	6	75.4-85.9	82.0	3.5	4.3
	0.5	6	66.1-91.1	77.7	10.0	12.8
	5.0	5	66.1-92.2	83.8	10.8	12.9
E-3	0.05	6	81.5-91.1	86.6	3.8	4.4
	0.5	6	61.7-91.4	72.6	11.8	16.3
	5.0	5	78.5-94.4	89.3	6.3	7.0

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Confirmation Ion Transition (Confirmation Method)						
Pyraflufen-ethyl	0.05	6	70.1-103	82.8	11.5	13.9
	0.5	6	61.4-95.3	73.0	13.7	18.8
	5.0	5	98.1-107	102	3.4	3.3
E-1	0.05	6	67.8-101	82.2	14.0	17.0
	0.5	6	72.9-84.6	79.1	4.8	6.1
	5.0	5	97.2-100	98.6	1.4	1.4
E-2	0.05	6	72.3-89.7	80.4	5.6	7.0
	0.5	6	63.9-91.4	76.6	10.4	13.6
	5.0	5	65.6-92.2	82.9	10.5	12.7
E-3	0.05	6	60.2-80.1	75.7	7.7	10.1
	0.5	6	60.0-90.8	72.3	12.3	17.1
	5.0	5	76.2-92.8	87.2	6.8	7.8

Data (uncorrected recovery results, p. 25) were obtained from pp. 13-15; Tables 7-14, pp. 36-43 of MRID 50336601.

1 Clay soil matrix [Lufa Speyer soil type 6S; 22% sand 27% silt 51% clay; pH 7.0 (0.01M CaCl₂), 1.7% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 86% sand 10% silt 4% clay; pH 6.0 (0.01M CaCl₂), 1.6% organic carbon] were used (USDA soil texture classification; p. 18; Appendices 8-9, pp. 87-88). Both soils were obtained from Battelle UK. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Analytes, except E-1, were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 413→339 and *m/z* 413→289 for pyraflufen-ethyl, *m/z* 327→277 and *m/z* 329→279 for E-2, and *m/z* 341→291 and *m/z* 341→276 for E-3. For E-1, only one ion transition was monitored: *m/z* 383→274; however, two LC/MS/MS systems were used, one for quantitation and one for confirmation.

Table 3. Independent Validation Method Recoveries for Pyraflufen-ethyl and its Metabolites E-1, E-2, and E-3 in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Clay Soil						
Quantitation Ion Transition						
Pyraflufen-ethyl	0.05	6	105.4-118.1	111.1	4.1	3.7
	0.5	6	109.2-116.8	112.4	2.8	2.5
	5.0	6	104.1-107.4	105.7	1.3	1.2
E-1	0.05	6	95.4-100.8	97.1	2.0	2.1
	0.5	6	87.0-96.8	93.6	4.5	4.8
	5.0	6	93.5-94.5	94.0	0.4	0.4
E-2	0.05	6	90.6-107.6	98.0	6.1	6.2
	0.5	6	95.5-112.4	103.8	5.7	5.5
	5.0	6	96.5-107.8	102.3	3.9	3.8
E-3	0.05	6	104.2-120.1	112.8	5.8	5.1
	0.5	6	109.5-118.1	113.5	3.0	2.7
	5.0	6	99.6-113.4	106.6	5.0	4.7
Confirmation Ion Transition (Confirmation Method)						
Pyraflufen-ethyl	0.05	6	100.1-137.9	113.7	13.0	11.4
	0.5	6	109.5-114.9	112.4	2.0	1.8
	5.0	6	103.8-109.8	106.1	2.1	2.0
E-1	0.05	6	87.9-98.9	92.4	3.8	4.1
	0.5	6	87.7-105.6	95.8	6.9	7.2
	5.0	6	94.5-100.3	96.8	2.3	2.4
E-2	0.05	6	88.4-109.9	101.3	8.8	8.7
	0.5	6	98.7-110.8	104.4	4.6	4.4
	5.0	6	95.9-109.4	102.8	4.5	4.4
E-3	0.05	6	99.0-121.0	110.3	8.2	7.4
	0.5	6	109.4-118.6	114.2	3.6	3.2
	5.0	6	102.4-115.7	108.0	4.9	4.5
Loamy Sand Soil						
Quantitation Ion Transition						
Pyraflufen-ethyl	0.05	6	98.0-106.0	101.8	3.1	3.0
	0.5	6	99.0-103.3	101.0	1.4	1.4
	5.0	6	93.1-97.2	95.7	1.4	1.5
E-1	0.05	6	90.0-97.7	93.3	2.6	2.8
	0.5	6	101.0-103.9	102.0	1.0	1.0
	5.0	6	99.6-103.7	101.8	1.6	1.6
E-2	0.05	6	93.8-112.6	104.1	8.2	7.9
	0.5	6	107.6-115.6	110.8	3.0	2.7
	5.0	6	101.3-107.6	105.0	2.3	2.2
E-3	0.05	6	98.5-121.3	108.5	8.0	7.4
	0.5	6	103.6-111.7	107.0	3.0	2.8
	5.0	6	98.5-102.8	100.4	1.5	1.5

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Confirmation Ion Transition (Confirmation Method)						
Pyraflufen-ethyl	0.05	6	100.7-107.7	103.4	2.9	2.8
	0.5	6	99.4-104.7	101.4	1.9	1.9
	5.0	6	93.0-97.2	95.4	1.4	1.5
E-1	0.05	6	70.2-90.3	81.0	7.0	8.7
	0.5	6	96.7-102.3	99.7	2.1	2.1
	5.0	6	92.3-101.7	98.8	3.6	3.6
E-2	0.05	6	78.8-106.9	93.0	11.5	12.4
	0.5	6	105.4-113.8	109.2	3.1	2.8
	5.0	6	100.2-108.1	104.1	2.9	2.8
E-3	0.05	6	111.4-127.5	118.0	6.1	5.2
	0.5	6	102.7-112.7	105.7	3.7	3.5
	5.0	6	97.2-100.9	99.3	1.5	1.5

Data (uncorrected recovery results, p. 19) were obtained from Tables 17-32, pp. 26-41 of MRID 50336602 and DER Attachment 2.

1 Clay soil matrix [Lufa Speyer soil type 6S; 25.6 ± 3.2% sand 34.4 ± 2.6% silt 40.0 ± 2.1% clay; pH 7.1 ± 0.5 (0.01M CaCl₂), 1.73 ± 0.05% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 ± 3.9% sand 16.3 ± 2.5% silt 7.9 ± 1.8% clay; pH 5.5 ± 0.1 (0.01M CaCl₂), 1.61 ± 0.15% organic carbon] were used (USDA soil texture classification; p. 13; Appendix B, pp. 113-115). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Analytes, except E-1, were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 413→339 and *m/z* 413→289 for pyraflufen-ethyl, *m/z* 327→277 and *m/z* 329→279 for E-2, and *m/z* 341→291 and *m/z* 341→276 for E-3. For E-1, only one ion transition was monitored with this LC/MS/MS system: *m/z* 383→274.

3 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). Rules of significant figures was followed when reporting results.

III. Method Characteristics

The LOQ for soil was 0.05 µg/kg in the ECM and ILV (pp. 15, 29-30; Table 5, p. 35 of MRID 50336601; pp. 10, 14-15, 42 of MRID 50336602). No justification of the LOQ was reported in the ECM or ILV. The LOD was reported as 0.01 µg/kg in the ECM, reportedly based on the lowest quantifiable calibration standard. In the ILV, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest mixed calibration standard (0.01 ng/mL; equivalent to 28% of the LOQ). The response of the lowest calibration standard was reportedly greater than three times the signal to noise for each mass transition. Specific calculations were not provided for the LOQ or LOD in the ECM or ILV, but the representative chromatograms appear to confirm adequate resolution of the analytes to meet analytical standards at these levels.

Table 4. Method Characteristics

Analyte		Pyraflufen-ethyl	E-1	E-2	E-3	
Limit of Quantitation (LOQ)		0.05 µg/kg				
Limit of Detection (LOD)	ECM	0.01 µg/kg				
	ILV	Not specified; less than 30% of the LOQ.				
Linearity (calibration curve r^2 coefficient of determination and concentration range) ¹	ECM	Clay	$r^2 = 0.9998$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9984$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9996$ (C)
		Loamy Sand	$r^2 = 0.9970$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9956$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9992$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9968$ (Q) $r^2 = 0.9976$ (C)
	ILV	Clay	$r^2 = 0.9988$ (Q) $r^2 = 0.9984$ (C)	$r^2 = 0.9988$ (Q) $r^2 = 0.9986$ (C)	$r^2 = 0.9984$ (Q) $r^2 = 0.9982$ (C)	$r^2 = 0.9978$ (Q & C)
		Loamy Sand	$r^2 = 0.9996$ (Q & C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)	$r^2 = 0.9992$ (Q) $r^2 = 0.9994$ (C)
	Concentration range		0.01-4.5 ng/mL			
Repeatable	ECM ^{2,3}	Yes at LOQ, 10×LOQ, and 100×LOQ.				
	ILV ^{2,4,5}					
Reproducible		Yes at LOQ, 10×LOQ, and 100×LOQ.				
Specific		Representative chromatograms of 10×LOQ and 100×LOQ were not provided. Minor baseline noise was noted in LOQ chromatograms.				
	ECM	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed in the loamy sand; matrix interferences were <i>ca.</i> 20% of the LOQ in clay in the C ion (based on peak area). ⁶	Yes, no matrix interferences were observed in the loamy sand; matrix interferences were <i>ca.</i> 10% of the LOQ in clay in the C ion (based on peak area). ⁶	Yes, in the loamy sand, no matrix interferences were observed. In clay soil, significant baseline noise was noted which interfered with peak attenuation ⁷ , and matrix interferences were <i>ca.</i> 5% of the LOQ in the C ion (based on peak area). ⁶	
	ILV	Yes, no matrix interferences were observed in the loamy sand; matrix interferences were <i>ca.</i> 10% of the LOQ in clay in the Q ion (based on peak area).	Yes, no matrix interferences were observed. Baseline noise was noted in the C ion. ⁶		Yes, no matrix interferences were observed in the loamy sand. In clay soil, baseline noise was noted which interfered with peak attenuation ⁷ , and matrix interferences were <i>ca.</i> 12% of the LOQ in clay in the Q ion (based on peak area).	

Data were obtained from pp. 13-15, 29-30; Tables 7-14, pp. 36-43 (recovery data); Figures 17-24, pp. 64-71 (calibration curve); Figures 1-16, pp. 48-63 (chromatograms) of MRID 50336601; pp. 10, 14-15, 42; Tables 17-32,

- pp. 26-41 (recovery data); Figures 2-60, pp. 50-108 (calibration curves & chromatograms) of MRID 50336602; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition or Confirmatory method.
- 1 Reported correlation coefficients of determination were reviewer-calculated from r values reported in the study report (Figures 17-24, pp. 64-71 of MRID 50336601; Figures 2-3, 8-9, 15, 20, 25, 30, 36-37, 42-43, 49-50, and 55-56, pp. 50-51, 56-57, 63, 68, 73, 78, 84-85, 90-91, 97-98, and 103-104 of MRID 50336602; DER Attachment 2). Matrix-matched standards were used in the ECM and the ILV.
 - 2 Analytes, except E-1, were identified using two ion transitions (quantitation and confirmation). For E-1, only one ion transition was monitored; however, two LC/MS/MS systems were used, one for quantitation and one for confirmation.
 - 3 In the ECM, clay soil matrix [Lufa Speyer soil type 6S; 22% sand 27% silt 51% clay; pH 7.0 (0.01M CaCl₂), 1.7% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 86% sand 10% silt 4% clay; pH 6.0 (0.01M CaCl₂), 1.6% organic carbon] were used (USDA soil texture classification; p. 18; Appendices 8-9, pp. 87-88 of MRID 50336601). Both soils were obtained from Battelle UK. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.
 - 4 In the ILV, clay soil matrix [Lufa Speyer soil type 6S; 25.6 ± 3.2% sand 34.4 ± 2.6% silt 40.0 ± 2.1% clay; pH 7.1 ± 0.5 (0.01M CaCl₂), 1.73 ± 0.05% organic carbon] and loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 ± 3.9% sand 16.3 ± 2.5% silt 7.9 ± 1.8% clay; pH 5.5 ± 0.1 (0.01M CaCl₂), 1.61 ± 0.15% organic carbon] were used (USDA soil texture classification; p. 13; Appendix B, pp. 113-115 of MRID 50336602). The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.
 - 5 Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method (pp. 10, 16-18, 20, 47 of MRID 50336602).
 - 6 A confirmatory method is not necessarily required when the primary method is LC/MS.
 - 7 Based on Figures 13-14, pp. 60-61 of MRID 50336601.
 - 8 Based on Figure 54, p. 102 of MRID 50336602.

IV. Method Deficiencies and Reviewer's Comments

1. The amount and details of the communication between the ILV testing facility and the method developer or ECM was not reported.
2. Representative chromatograms of 10×LOQ and 100×LOQ fortifications were not provided. Chromatograms from all fortifications and matrices should be provided for review to assess the specificity of the method.
3. The ECM and ILV matrices were both clay soil and loamy sand soil (p. 18; Appendices 8-9, pp. 87-88 of MRID 50336601; p. 13; Appendix B, pp. 113-115 of MRID 50336602). The soil characterizations of the matrices showed that these soils were similar, but not the same. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method, based on percent organic carbon, and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies.
4. The specific number of ILV trials required to validate the method was not reported. This reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method. (See pp. 10, 16-18, 20, 47 of MRID 50336602).
5. The estimation procedure for the LOQ and LOD in ECM and ILV was not fully described as specified in 40 CFR Part 136 (pp. 15, 29-30; Table 5, p. 35 of MRID 50336601; pp. 10, 14-15, 42 of MRID 50336602). In the ECM, the LOD was reportedly based on the lowest quantifiable calibration standard. In the ILV, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest mixed calibration

standard (0.01 ng/mL; equivalent to 28% of the LOQ). The response of the lowest calibration standard was reportedly greater than three times the signal to noise for each mass transition. No specific value was reported in the ILV. The representative chromatograms provided at or near the LOD did appear to confirm adequate resolution suitable for quantification of the analytes.

6. The ECM matrices of clay and loamy sand were referred to as clay and sandy, respectively, in the study report (p. 18; Appendices 8-9, pp. 87-88 of MRID 50336601). The ILV matrices of clay and loamy sand were referred to as clayey loam and loamy sand, respectively, in the study report based on the German DIN classification (p. 13; Appendix B, pp. 113-115 of MRID 50336602). The reviewer referred to the soils based on their USDA soil texture classification in the DER and DER Attachments.
7. In the ECM and ILV, the matrix effects were evaluated and found to be significant; matrix-matched standards were used (p. 21; Tables 1-4, pp. 31-34 of MRID 50336601; p. 22; Tables 1-16, pp. 21-25 of MRID 50336602).
8. The extract and stock solution stabilities were evaluated in the ECM (pp. 44-47; Tables 15-16, pp. 44-47 of MRID 50336601). When refrigerated (4°C), the stock solutions of all analytes were not found to be stable after 42 days. When refrigerated (4°C), the extract solutions of E-2 and E-3 were found to be stable after 35 days; extract solutions of pyraflufen-ethyl and E-1 were not found to be stable after 35 days.

The extract and stock solution stabilities were evaluated in the ILV (pp. 42, 46; Tables 33-44, pp. 42-46 of MRID 50336602). When refrigerated (2 to 8°C), the extract solutions of all analytes were found to be stable up to 10 days. When refrigerated (2 to 8°C), the stock solutions were found to be stable up to 15 days.

9. In the ECM, the time required to complete the extraction of one set of 21 samples (one reagent blank, two matrix controls and 18 fortified samples) and preparation of eight calibration standard was reported as *ca.* 7.5 hours, followed by *ca.* 15 hours for LC/MS/MS analysis (p. 24 of MRID 50336601). The total time requirement of the method was reported as *ca.* 2.5 working days in the ECM. In the ILV, the time required to complete the extraction of one set of 21 samples (one reagent blank, two matrix controls and 18 fortified samples) and preparation of eight calibration standard was reported as *ca.* 7.5 hours, followed by *ca.* 20 hours for LC/MS/MS analysis (p. 20 of MRID 50336602). The total time requirement of the method was reported as *ca.* 2 working days in the ILV.

V. References

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

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

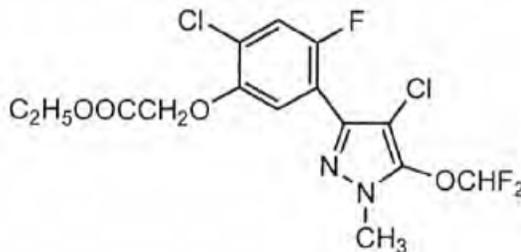
DER Attachment 1: Chemical Names and Structures**Pyraflufen-ethyl**

IUPAC Name: Ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate

CAS Name: 129630-19-9

CAS Number: Not reported

SMILES String: Not found

**E-1**

IUPAC Name: 2-Chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found



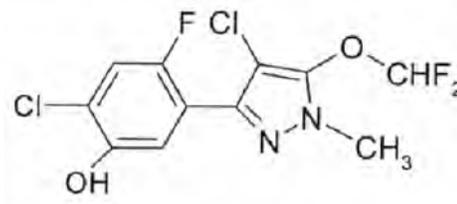
E-2

IUPAC Name: 2-Chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenol

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found

**E-3**

IUPAC Name: 4-Chloro-3-(4-chloro-2-fluoro-5-methoxyphenyl)-5-difluoromethoxy-1-methylpyrazole

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found

