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

Reports: ECM: EPA MRID No.: 50414201. Coleman, H. 2017. Method Validation for the Determination of Residues of Pyraflufen-ethyl and Metabolites E-1, E-2 and E-3 in Water. Study No.: XG/17/007. 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; 101 pages. Final report issued June 20, 2017.

ILV: EPA MRID No. 50414202. Watson, G. 2017. Independent Laboratory Validation of analytical method XG/17/007 for the determination of residues of pyraflufen-ethyl and metabolites E-1, E-2 and E-3 in water by LC-MS/MS. Study Reference No.: RES-00109. 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; 106 pages (including page 2a). Final report issued August 16, 2017.

Document No.: MRIDs 50414201 & 50414202

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 15, p. 101 of MRID 50414201). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 15, p. 101). 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. 2a; Appendix C, p. 106 of MRID 50414202). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2, 2a, 4; Appendix C, p. 106). 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 study sponsor was not detailed. The ILV study authors noted only that "After consultation with the sponsor, the validation batches were reconstituted using acetonitrile/water (10/90, v/v) in order to improve recovery levels". In order for the ECM to potentially to 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 fortification levels were provided.

PC Code: 030090

EFED Final Reviewer:	Dena Barrett, Chemist	Signature: Dena Barrett Date: 9/17/20
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature: Lisa Muto Date: 1/11/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: Kathleen P. Ferguson 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/17/007, is designed for the quantitative determination of pyraflufen-ethyl and it metabolites E-1, E-2, and E-3 in water at the LOQ of 0.01 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for all analytes. The ECM and ILV used characterized drinking and surface water matrices; matrices were not the same. All analytes were identified using two ion transitions. All submitted ILV and ECM data pertaining to precision, repeatability, and reproducibility was acceptable. The linearity data of the ILV was acceptable; however, quadratic regression was used for pyraflufen-ethyl. The linearity data of the ECM was unacceptable for pyraflufen-ethyl and E-1 in one or both matrices. The specificity of the method was determined to be acceptable for all analytes in both matrices in the ECM and ILV, except for ECM chromatograms of pyraflufen-ethyl; however, representative chromatograms of 10×LOQ and 100×LOQ fortifications were not provided. The LOD was not reported in the ILV.

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	50414201[1]	50414202[2]		Water	20/06/2017	Nichino America, Inc.	LC/MS/MS	0.01 µg/L
E-1								
E-2								
E-3								

1 In the ECM, drinking water matrix (15/002; pH 8.1, 3.7 mg/L dissolved organic carbon, 299 mg hardness as $CaCO_3$/L), obtained from a drinking water tap at Battelle UK Test Facility, Essex, United Kingdom, and surface water matrix (16/068; pH 8.0, 3.4 mg/L dissolved organic carbon, 127 mg hardness as $CaCO_3$/L), obtained from Carsington Lake - Millfields, were used (p. 19; Appendices 9-10, pp. 93-94 of MRID 50414201). Both waters were obtained from Battelle UK. The water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, drinking water matrix (RES-00109; pH 8.0, 3.23 mg/L dissolved organic carbon, 209 mg/L total hardness as $CaCO_3$), obtained from a drinking water tap at Derwent Business Centre, Derby, United Kingdom,

and surface water matrix (RES-00109; pH 8.5, 9.58 mg/L dissolved organic carbon, 260 mg/L total hardness as $CaCO_3$), obtained from a lake at Attenborough Nature Reserve, Nottingham, United Kingdom, were used (p. 12; Appendix B, pp. 103-104 of MRID 50414202). The water characterization was performed by CEMAS.

I. Principle of the Method

For pyraflufen-ethyl, E-2, and E-3, water samples (15 mL) were fortified with fortification solutions of pyraflufen-ethyl, E-2, and E-3 in glass vials (pp. 21, 24; Appendix 1, p. 85 of MRID 50414201). The samples were extracted three times with ethyl acetate (3 x 5 mL) via vigorous shaking for 30 seconds. The combined extracts were reduced to dryness under a stream of nitrogen in a heating block set at 40°C. After the residue was reconstituted in 2 mL of water via sonication and vortex mixing, an aliquot of the sample was transferred to an autosampler vial and analyzed by HPLC/MS/MS.

For E-1, water samples (10 mL) were fortified with fortification solutions of E-1 in glass vials (pp. 21, 25; Appendix 2, p. 86 of MRID 50414201). After shaking, 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 6500 MS equipped with a Zorbax SB-C3 column (3.0 mm x 150 mm, 5.0 µm; column temperature 20°C) using a mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [percent A:B at 0.0 min. 90:10, 4.0-5.0 min. 5:95, 5.1-6.5 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. 25-27; Appendix 3, pp. 87-88 of MRID 50414201). Injection volume was 95 µL. Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 413→339 and *m/z* 413→289 for pyraflufen-ethyl, *m/z* 383→274 and *m/z* 385→276 for E-1, *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. Expected retention times were *ca*. 3.9-4.1, 3.4-3.6, 3.5-3.6, and 3.7-4.0 minutes for pyraflufen-ethyl, E-1, E-2, and E-3, respectively.

In the ILV, the ECM was performed as written, except that the pyraflufen-ethyl, E-2, and E-3 sample residues were reconstituted in acetonitrile:water (10:90, v:v) and insignificant modifications were made to the analytical parameters (pp. 10, 12, 15-18, 20-22 of MRID 50414202). For analyte identification, an Agilent 1290 Series HPLC coupled to an AB Sciex API 5500 MS was used and equipped with a Zorbax SB-C3 column (3.0 mm x 150 mm, 5.0 µm; column temperature 50°C) using a mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [percent A:B at 0.0 min. 80:20, 4.0-5.0 min. 5:95, 5.1-6.5 min. 80:20] 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 MRM. Injection volume was 60 µL. Pyraflufen-ethyl, E-1, E-2, and E-3 were identified using the same ion transitions. Expected retention times were *ca*. 3.7, 3.1, 3.2, and 3.6 minutes for pyraflufen-ethyl, E-1, E-2, and E-3, respectively. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for water was 0.01 µg/L in the ECM and ILV (pp. 15-16 of MRID 50414201; pp. 9, 14, 19, 44 of MRID 50414202). In the ECM, the Limit of Detection

(LOD) was reported as 0.002 µg/L for all analytes/ions/matrices, except for the confirmation ion transition of pyraflufen-ethyl in surface water, which was reported as 0.003 µg/L. In the ILV, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest mixed calibration standard (0.003 ng/mL for E-1 and 0.0225 ng/mL for all other analytes; equivalent to 30% of the LOQ).

II. Recovery Findings

ECM (MRID 50414201): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of pyraflufen-ethyl, E-1, E-2, and E-3 at fortification levels of 0.01 µg/L (LOQ), 0.1 µg/L (10×LOQ), and 1.0 µg/L (100×LOQ) in two water matrices (pp. 14-15; Tables 7-14, pp. 42-49). All analytes were identified using two ion transitions. Performance data (recovery results) from quantitation and confirmation analyses were comparable. Drinking water matrix (15/002; pH 8.1, 3.7 mg/L dissolved organic carbon, 299 mg hardness as $CaCO_3$/L), obtained from a drinking water tap at Battelle UK Test Facility, Essex, United Kingdom, and surface water matrix (16/068; pH 8.0, 3.4 mg/L dissolved organic carbon, 127 mg hardness as $CaCO_3$/L), obtained from Carsington Lake - Millfields, were used (p. 19; Appendices 9-10, pp. 93-94). Both waters were obtained from Battelle UK. The water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50414202): Mean recoveries and RSDs were within guideline requirements for analysis of pyraflufen-ethyl, E-1, E-2, and E-3 at fortification levels of 0.01 µg/L (LOQ), 0.1 µg/L (10×LOQ), and 1.0 µg/L (100×LOQ) in two water matrices (Tables 17-32, pp. 26-41). All analytes were identified using two ion transitions. Performance data (recovery results) from quantitation and confirmation analyses were comparable. drinking water matrix (RES-00109; pH 8.0, 3.23 mg/L dissolved organic carbon, 209 mg/L total hardness as $CaCO_3$), obtained from a drinking water tap at Derwent Business Centre, Derby, United Kingdom, and surface water matrix (RES-00109; pH 8.5, 9.58 mg/L dissolved organic carbon, 260 mg/L total hardness as $CaCO_3$), obtained from a lake at Attenborough Nature Reserve, Nottingham, United Kingdom, were used (p. 12; Appendix B, pp. 103-104 of MRID 50414202). The water characterization was performed by CEMAS. The method was validated after the second trial with the modification of the pyraflufen-ethyl, E-2, and E-3 reconstitution solvent and insignificant modifications to the analytical method (pp. 10, 12, 15-18, 20-22).

Table 2. Initial Validation Method Recoveries for Pyraflufen-ethyl and its Metabolites E-1, E-2, and E-3 in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation Ion Transition					
Pyraflufen-ethyl	0.01	6	69.3-97.9	81.4	11.9	14.6
	0.1	6	82.8-113	93.9	11.9	12.7
	1.0	6	82.3-129	104	15.8	15.3
E-1	0.01	6	71.9-102	89.6	11.5	12.9
	0.1	6	89.7-93.6	91.5	1.4	1.6
	1.0	6	88.9-92.1	91.1	1.2	1.3
E-2	0.01	6	70.3-81.2	76.6	5.7	7.4
	0.1	6	76.9-92.7	85.7	5.8	6.7
	1.0	6	87.9-101	94.8	4.8	5.1
E-3	0.01	6	72.0-77.7	74.6	2.2	3.0
	0.1	6	77.6-90.7	82.5	4.4	5.3
	1.0	6	86.4-99.3	92.8	5.7	6.1
	Confirmation Ion Transition					
Pyraflufen-ethyl	0.01	6	71.2-114	92.7	14.2	15.3
	0.1	6	77.7-122	99.1	18.3	18.5
	1.0	6	81.6-129	105	18.4	17.5
E-1	0.01	6	77.2-118	95.0	15.8	16.6
	0.1	6	86.7-94.8	90.5	2.6	2.9
	1.0	6	88.9-94.1	91.7	1.7	1.8
E-2	0.01	6	70.4-84.4	79.6	5.4	6.8
	0.1	6	75.5-89.1	83.4	5.4	6.4
	1.0	6	86.1-101	93.3	5.6	6.0
E-3	0.01	6	63.1-93.6	79.3	11.0	13.8
	0.1	6	81.2-89.9	84.0	3.4	4.1
	1.0	6	85.6-100	94.2	6.3	6.6
	Surface Water					
	Quantitation Ion Transition					
Pyraflufen-ethyl	0.01	6	74.3-98.4	88.3	10.3	11.6
	0.1	6	81.1-104	95.8	8.0	8.3
	1.0	5	63.1-85.9	77.9	9.0	11.6
E-1	0.01	6	69.6-82.7	75.8	4.7	6.1
	0.1	6	85.8-94.7	88.6	3.2	3.7
	1.0	6	90.1-92.4	91.1	0.9	1.0
E-2	0.01	6	77.2-103	95.6	9.4	9.8
	0.1	6	98.7-113	105	5.1	4.9
	1.0	5	96.3-113	103	6.4	6.2
E-3	0.01	6	72.8-101	91.6	10.5	11.5
	0.1	6	99.5-109	104	3.0	2.9
	1.0	6	66.5-105	92.0	14.0	15.3

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmation Ion Transition					
Pyraflufen-ethyl	0.01	6	66.3-95.5	82.9	12.2	14.7
	0.1	6	86.1-106	98.3	7.3	7.4
	1.0	5	63.2-87.3	78.6	9.6	12.2
E-1	0.01	6	68.8-79.7	72.6	3.9	5.4
	0.1	6	87.1-96.3	90.9	3.3	3.6
	1.0	6	88.8-92.0	90.8	1.2	1.3
E-2	0.01	6	72.8-101	92.9	10.6	11.4
	0.1	6	93.2-113	101	7.1	7.0
	1.0	5	95.5-109	101	5.1	5.1
E-3	0.01	6	73.2-94.0	86.0	6.8	8.0
	0.1	6	94.7-106	97.6	4.2	4.3
	1.0	6	63.3-97.6	86.2	13.1	15.2

Data (uncorrected recovery results, p. 29) were obtained from pp. 14-15; Tables 7-14, pp. 42-49 of MRID 50414201.

1 Drinking water matrix (15/002; pH 8.1, 3.7 mg/L dissolved organic carbon, 299 mg hardness as $CaCO_3$/L), obtained from a drinking water tap at Battelle UK Test Facility, Essex, United Kingdom, and surface water matrix (16/068; pH 8.0, 3.4 mg/L dissolved organic carbon, 127 mg hardness as $CaCO_3$/L), obtained from Carsington Lake - Millfields, were used (p. 19; Appendices 9-10, pp. 93-94). Both waters were obtained from Battelle UK. The water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 413→339 and *m/z* 413→289 for pyraflufen-ethyl, *m/z* 383→274 and *m/z* 385→276 for E-1, *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.

Table 3. Independent Validation Method Recoveries for Pyraflufen-ethyl and its Metabolites E-1, E-2, and E-3 in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation Ion Transition					
Pyraflufen-ethyl	0.01	6	94.4-96.8	95.4	1.1	1.1
	0.1	6	89.1-96.5	93.3	2.5	2.7
	1.0	6	92.5-96.2	94.8	1.4	1.5
E-1	0.01	6	95.2-107.3	98.9	4.4	4.5
	0.1	6	96.2-106.4	101.8	3.6	3.5
	1.0	6	99.8-104.4	101.0	1.7	1.7
E-2	0.01	6	96.4-98.4	97.3	0.7	0.8
	0.1	6	102.9-108	105.2	1.8	1.7
	1.0	6	98.7-101.6	99.8	1.1	1.1
E-3	0.01	6	92.3-98.3	95.5	2.2	2.3
	0.1	6	105-112.1	109.0	2.7	2.5
	1.0	6	99.5-101.6	100.5	0.7	0.7
	Confirmation Ion Transition					
Pyraflufen-ethyl	0.01	6	96.5-100	98.4	1.6	1.6
	0.1	6	91.7-100.7	96.2	3.3	3.5
	1.0	6	92.2-98.3	95.2	2.5	2.6
E-1	0.01	6	93.7-109.8	100.5	6.6	6.5
	0.1	6	97.9-105.8	102.5	2.8	2.7
	1.0	6	98.1-104.6	100.9	2.2	2.2
E-2	0.01	6	95.4-99.3	97.7	1.4	1.5
	0.1	6	101.1-104.8	103.0	1.2	1.2
	1.0	6	96.4-100.3	99.0	1.4	1.5
E-3	0.01	6	91.6-94.0	93.2	0.9	1.0
	0.1	6	96.4-99.7	98.5	1.3	1.3
	1.0	6	93.2-96.2	94.8	1.3	1.3
	Surface Water					
	Quantitation Ion Transition					
Pyraflufen-ethyl	0.01	6	93.7-99.8	96.1	2.1	2.2
	0.1	6	88.1-95.5	92.8	2.6	2.8
	1.0	6	87.8-98.5	91.5	4.6	5.0
E-1	0.01	6	91.3-97.1	94.2	2.0	2.1
	0.1	6	97.4-103.3	100.0	2.4	2.4
	1.0	6	94.0-97.9	96.1	1.6	1.6
E-2	0.01	6	98.9-100.8	99.7	0.7	0.7
	0.1	6	102.7-105.8	104.4	1.2	1.2
	1.0	6	96.9-100.9	99.0	1.4	1.4
E-3	0.01	6	95.2-101.0	98.3	2.1	2.2
	0.1	6	107.2-110.2	108.3	1.1	1.1
	1.0	6	97.6-101.3	98.9	1.3	1.4

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Confirmation Ion Transition					
Pyraflufen-ethyl	0.01	6	93.5-98.8	96.3	2.0	2.1
	0.1	6	93.1-98.7	95.2	2.3	2.4
	1.0	6	87.1-97.7	91.8	4.4	4.8
E-1	0.01	6	93.0-105.4	98.1	5.0	5.1
	0.1	6	98.6-104.2	101.3	1.8	1.8
	1.0	6	96.1-98.6	97.3	1.0	1.0
E-2	0.01	6	92.9-103.0	97.1	3.7	3.8
	0.1	6	99.3-103.3	101.0	1.5	1.5
	1.0	6	94.6-97.8	96.7	1.2	1.2
E-3	0.01	6	93.7-101.0	97.0	2.7	2.7
	0.1	6	97.9-99.9	99.3	0.8	0.8
	1.0	6	94.2-95.8	94.6	0.6	0.6

Data (uncorrected recovery results, pp. 16-17, 21) were obtained from Tables 17-32, pp. 26-41 of MRID 50414202 and DER Attachment 2.

1 Drinking water matrix (RES-00109; pH 8.0, 3.23 mg/L dissolved organic carbon, 209 mg/L total hardness as $CaCO_3$), obtained from a drinking water tap at Derwent Business Centre, Derby, United Kingdom, and surface water matrix (RES-00109; pH 8.5, 9.58 mg/L dissolved organic carbon, 260 mg/L total hardness as $CaCO_3$), obtained from a lake at Attenborough Nature Reserve, Nottingham, United Kingdom, were used (p. 12; Appendix B, pp. 103-104). The water characterization was performed by CEMAS.

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 413→339 and *m/z* 413→289 for pyraflufen-ethyl, *m/z* 383→274 and *m/z* 385→276 for E-1, *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.

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 water was 0.01 µg/L in the ECM and ILV (pp. 15-16; Table 5, p. 39 of MRID 50414201; pp. 9, 14, 19, 44 of MRID 50414202). The LOQ was set at 3 to 5 times the LOD. In the ECM, the LOD was reportedly based on the lowest quantifiable calibration standard. In the ECM, the LOD was reported as 0.002 µg/L for all analytes/ions/matrices, except for the confirmation ion transition of pyraflufen-ethyl in surface water, which was reported as 0.003 µg/L. In the ILV, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest mixed calibration standard (0.003 ng/mL for E-1 and 0.0225 ng/mL for all other analytes; equivalent to 30% 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 calculations were provided for the LOQ or LOD in the ECM or ILV.

Table 4. Method Characteristics

Analyte			Pyraflufen-ethyl	E-1	E-2	E-3
Limit of Quantitation (LOQ)			0.01 µg/L			
Limit of Detection (LOD)	ECM	Drinking	0.002 µg/L (Q & C)	0.002 µg/L (Q & C)		
		Surface	0.002 µg/L (Q) 0.003 µg/L (C)			
	ILV		Not specified; less than 30% of the LOQ.			
Linearity (calibration curve r^2 coefficient of determination and concentration range)[1]	ECM	Drinking	r^2 = **0.9948** (Q) r^2 = **0.9938** (C)	r^2 = **0.9934** (Q) r^2 = **0.9938** (C)	r^2 = 0.9994 (Q) r^2 = 0.9978 (C)	r^2 = 0.9970 (Q) r^2 = 0.9982 (C)
		Surface	r^2 = **0.9896** (Q) r^2 = **0.9878** (C)	r^2 = 0.9974 (Q) r^2 = 0.9976 (C)	r^2 = 0.9986 (Q) r^2 = 0.9976 (C)	r^2 = 0.9984 (Q) r^2 = **0.9932** (C)
	Concentration range		0.015-12.5 ng/mL	0.002-1.5 ng/mL	0.015-12.5 ng/mL	
	ILV	Drinking	r^2 = 1.0000 (Q & C)[2]	r^2 = 0.9998 (Q) r^2 = 1.0000 (C)[1]	r^2 = 0.9980 (Q) r^2 = 0.9990 (C)	r^2 = 0.9956 (Q) r^2 = 0.9998 (C)
		Surface	r^2 = 0.9998 (Q)[2] r^2 = 1.0000 (C)[2]		r^2 = 0.9986 (Q) r^2 = 0.9994 (C)	r^2 = 0.9956 (Q) r^2 = 0.9998 (C)
	Concentration range		0.0225-12.5 ng/mL	0.003-1.5 ng/mL	0.0225-12.5 ng/mL	
Repeatable[3]	ECM[4]		Yes, at LOQ, 10×LOQ, and 100×LOQ.			
	ILV[5,6]					
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, matrix interferences were *ca*. 7-8% of the LOQ in the Q ion (based on peak area). In drinking water, significant baseline noise was noted which interfered with peak attenuation.[7] In drinking water, matrix interferences were *ca*. 27% of the LOQ in the C ion (based on peak area).[8]	Matrix interferences were *ca*. 10-23% of the LOQ in the Q ion (based on peak area).[9] In drinking water, baseline noise was noted which interfered with peak attenuation.[9] In drinking water, matrix interferences were *ca*. 18% of the LOQ in the C ion (based on peak area).[8,9]	Yes, matrix interferences were *ca*. 2% of the LOQ (based on peak area).	Yes, matrix interferences were *ca*. 2-8% of the LOQ (based on peak area).
	ILV		Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Baseline noise was noted which interfered with peak attenuation.[10]	Yes, no matrix interferences were observed.	

Data were obtained from pp. 14-16, 24; Tables 7-14, pp. 42-49 (recovery data); Figures 17-24, pp. 70-77 (calibration curve); Figures 1-16, pp. 54-69 (chromatograms) of MRID 50414201; pp. 9, 14, 19, 44; Tables 17-32, pp. 26-41 (recovery data); Figures 2-60, pp. 52-98 (calibration curves & chromatograms) of MRID 50414202; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.
Coefficient of determination (r^2) values <0.995 are in **red** text.

1 Reported correlation coefficients of determination were reviewer-calculated from r values reported in the study report (p. 24; Figures 17-24, pp. 70-77 of MRID 50414201; Figures 2-3, 8-9, 15-16, 24-25, 30-31, 37-38, and 43-44, pp. 52-53, 58-59, 65-66, 74-75, 80-81, 87-88, and 93-94 of MRID 50414202; DER Attachment 2). In the ECM and ILV, matrix-matched standards were used for pyraflufen-ethyl, E-2 and E-3; solvent standards were used for E-1.

2 Quadratic regression was used.

3 All analytes were identified using two ion transitions (quantitation and confirmation.

4 In the ECM, drinking water matrix (15/002; pH 8.1, 3.7 mg/L dissolved organic carbon, 299 mg hardness as $CaCO_3$/L), obtained from a drinking water tap at Battelle UK Test Facility, Essex, United Kingdom, and surface water matrix (16/068; pH 8.0, 3.4 mg/L dissolved organic carbon, 127 mg hardness as $CaCO_3$/L), obtained from Carsington Lake - Millfields, were used (p. 19; Appendices 9-10, pp. 93-94 of MRID 50414201). Both waters were obtained from Battelle UK. The water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

5 In the ILV, drinking water matrix (RES-00109; pH 8.0, 3.23 mg/L dissolved organic carbon, 209 mg/L total hardness as $CaCO_3$), obtained from a drinking water tap at Derwent Business Centre, Derby, United Kingdom, and surface water matrix (RES-00109; pH 8.5, 9.58 mg/L dissolved organic carbon, 260 mg/L total hardness as $CaCO_3$), obtained from a lake at Attenborough Nature Reserve, Nottingham, United Kingdom, were used (p. 12; Appendix B, pp. 103-104 of MRID 50414202). The water characterization was performed by CEMAS.

6 The method was validated after the second trial with the modification of the pyraflufen-ethyl, E-2, and E-3 reconstitution solvent and insignificant modifications to the analytical method (pp. 10, 12, 15-18, 20-22 of MRID 50414202).

7 Based on Figures 1-2, pp. 54-55 of MRID 50414201.

8 A confirmatory method is not necessarily required when the primary method is LC/MS.

9 Based on Figures 5-8, pp. 58-61 of MRID 50414201.

10 Based on Figures 17-22, pp. 67-72 of MRID 50414202.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, the linearity of the pyraflufen-ethyl dose response curve was slightly below 0.995 in drinking water [Coefficient of determination or $r^2 = 0.9948$ (Q), $r^2 = 0.9938$ (C)] and surface water [$r^2 = 0.9896$ (Q), $r^2 = 0.9878$ (C)], E-1 in drinking water [$r^2 = 0.9934$ (Q), $r^2 = 0.9938$ (C)], and confirmation ion of E-3 in surface water [$r^2 = 0.9932$ (C); Figures 17-24, pp. 70-77 of MRID 50414201; DER Attachment 2]. Quadratic regression (as was performed for the ILV) would be expected to provide higher coefficients of determination Note that a confirmatory method is not necessarily required when the primary method is LC/MS.

   In the ILV, quadratic regression was used for pyraflufen-ethyl (pp. 9, 16, 27; Figures 2-3, pp. 52-53; Figures 8-9, pp. 58-59 of MRID 50414202). Quadratic regression always gave r^2 values > 0.995.

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 representative chromatograms of pyraflufen-ethyl in drinking water showed significant baseline noise which interfered with peak attenuation (Figures 1-2, pp. 54-55 of MRID 50414201). Additionally, ECM representative chromatograms showed matrix interferences in the quantitation ion which were *ca*. 10-23% of the LOQ (based on peak area; Figures 5-8, pp. 58-61).

4. The estimation procedure for the LOQ and LOD in ECM and ILV was not fully described as specified in 40 CFR Part 136 (pp. 15-16; Table 5, p. 39 of MRID 50414201; pp. 9, 14, 19, 44 of MRID 50414202). 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 (0.003 ng/mL for E-1 and 0.0225 ng/mL for all other analytes; equivalent to 30% 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.

5. The communication between the ILV testing facility and the study sponsor was not detailed; it was only reported that the modifications which were made to the method were the result of consultation with the study sponsor (p. 10 of MRID 50414202).

6. In the ECM, the matrix effects were evaluated and found to be significant for pyraflufen-ethyl, E-2 and E3; matrix-matched standards were used (p. 30; Tables 1-4, pp. 35-38 of MRID 50414201). Solvent standards were used for E-1. In the ILV, matrix effects were not found to be significant, but calibration standards were prepared for pyraflufen-ethyl, E-2 and E-3 in order to be consistent with the ECM (p. 22; Tables 1-16, pp. 23-26 of MRID 50414202).

   The extract and stock solution stabilities were evaluated in the ECM (pp. 50-53; Tables 15-16, pp. 50-53 of MRID 50414201). When refrigerated (4°C), the stock solutions of all analytes, except E-1, were not found to be stable after 40 days. When refrigerated (4°C), the extract solutions of E-2 and E-3 were found to be stable after 22 days; extract solutions of pyraflufen-ethyl and E-1 were found to be stable after 14-15 days for drinking water, but not surface water.

   The extract and stock solution stabilities were evaluated in the ILV (pp. 44, 47; Tables 33-44, pp. 44-48 of MRID 50414202). When refrigerated (2 to 8°C), the extract solutions of all analytes were found to be stable up to 7 days. When refrigerated (2 to 8°C), the stock solutions were found to be stable up to 21-26 days.

7. 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*. 3.5 hours, followed by *ca*. 11 hours (pyraflufen-ethyl, E-2 and E-3) and *ca*. 7.5 hours (E-1) for LC/MS/MS analysis (p. 28 of MRID 50414201). 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*. 12 hours (pyraflufen-ethyl, E-2 and E-3) and *ca*. 10 hours (E-1) for LC/MS/MS analysis (p. 18 of MRID 50414202).

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

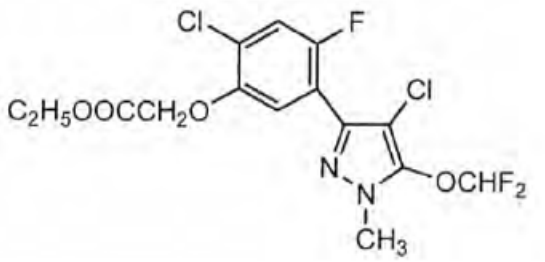
Pyraflufen-ethyl

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



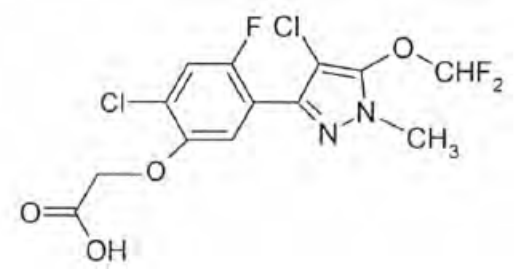
E-1

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
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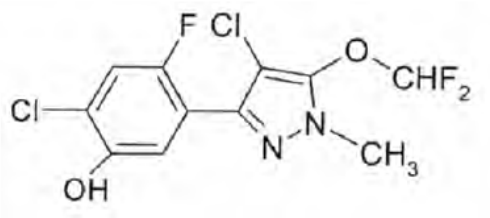

E-3

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