Analytical method for fluroxypyr-MHE and and its metabolites, fluroxypyr acid, fluroxypyr-DCP and fluroxypyr-MP, in water

Reports: ECM: EPA MRID No. 50290803 (appendix a): Dow AgroSciences Study No. 081042 (DAS# 081042). Hill, R.C. 2017. Validation Report for the Determination of Residues of Fluroxypyr 1-Methylheptyl Ester, Fluroxypyr and its Major Metabolites in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study No.: 081042. Report prepared, sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 113 pages (including amendment pages). Final report issued January 15, 2010; 1st Amendment dated February 16, 2010; 2nd Amendment dated March 6, 2017.

ILV: EPA MRID No. 50290803. Austin, R., N.W. Rawle. 2017. Independent Laboratory Validation of a Method for the Determination of Residues of Fluroxypyr 1-Methylheptyl Ester, Fluroxypyr and its Major Metabolites in Surface Water, Ground Water and Drinking Water. Dow AgroSciences Study Reference No.: 101083. Study ID: CEMS-4626. Report prepared by CEM Analytical Services Ltd. (CEMAS), Berkshire, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 112 pages. Final report issued March 24, 2010; Report Version 2 Issued May 11, 2017.

Document No.: MRID 50290803

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) and OECD Good Laboratory Practices (GLP) standards (p. 3R2 of DAS# 081042). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2R2-4R2). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4R2).

ILV: The study was conducted in accordance with OECD and UK GLP standards (p. 3; Appendix C, p. 111 of MRID 50290803). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix C, p. 111). 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 **UNACCEPTABLE <u>but upgradable</u>**. For the application of the method to fluroxypyr-MHE, an updated ECM should be submitted to include the ILV modifications. The ECM contained many deficiencies, including unsatisfactory performance data for fluroxypyr-MHE for several matrices/fortifications, insufficient number of samples prepared at 10×LOQ, insufficient support for the specificity of the method for fluroxypyr-MHE, incomplete chromatographic support, and uncharacterized matrices.

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EFED Final Reviewer:	Rochelle F. H. Bohaty, PhD, Senior Chemist	Signature: ROCHELLE BOHATY Digitally signed by ROCHELLE BOHATY Date: 2019.09.12 11:21:22 -04'00' Date: September 12, 2019
	Sarah Hafner, PhD, Chemist	Signature: Date: September 12, 2019
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S. Environmental Scientist	Signature: Date: 2/15/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: Date: 2/15/18

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Dow AgroSciences Study No. 081042 (DAS# 081042), is designed for the quantitative determination of fluroxypyr-MHE and its metabolites, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, in water with a LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern[1] in water for all four analytes. The ECM performed the method using uncharacterized surface, ground, and drinking water matrices; the ILV validated the method using characterized surface, ground, and drinking water matrices. The ILV validated the method for all four analytes in the first trial with the use of three or four 2.0 mL aliquots of acetonitrile to elude the analytes from the Strata-X SPE, instead of two aliquots, and insignificant modifications to the analytical instrumentation. All ILV data regarding the linearity, repeatability, and specificity of the method was satisfactory for all four analytes in all three matrices. The ECM contained many deficiencies, including unsatisfactory performance data for fluroxypyr-MHE for several matrices/fortifications, insufficient number of samples prepared at 10×LOQ (n = 3), insufficient support for the specificity of the method for fluroxypyr-MHE (matrix interferences were *ca*. 10-39%), and incomplete chromatographic support (10×LOQ and LOD not presented). For the application of the method to fluroxypyr-MHE, an updated ECM should be submitted to include the ILV modifications**.**

[1] 0.09 mg/L vascular 30-day IC_{50} (Mohr et al., 2013)

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						
Fluroxypyr-MHE	DAS# 081042	50290803		Water[2,3]	20/02/2017	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L
Fluroxypyr acid								
Fluroxypyr-DCP								
Fluroxypyr-MP								

1 Fluroxypyr MHE = Fluroxypyr 1-MHE, Fluroxypyr 1-methylheptyl ester; Fluroxypyr-DCP = Fluroxypyr dichloropyridinol; and Fluroxypyr-MP = Fluroxypyr methoxypyridine.

2 In the ECM, three water matrices were used. Loam water (M964; 49% sand 40% silt 11% clay, pH 7.2, 0.7% organic carbon) obtained from Hanford, (location nor further specified), silt loam water (M961; 35% sand 57% silt 8% clay, pH 5.8, 3.9% organic carbon) obtained from Brierlow, (location nor further specified), unclassified water (M963; uncharacterized) from an unidentified location, sandy loam water (M971; 69% sand 18% silt 13% clay, pH 5.9, 2.9% organic carbon) obtained from Emperor Lake (location nor further specified), and sand water (M972; 97% sand 3% silt 0% clay, pH 7.7, 1.0% organic carbon) obtained from River Dove, (location nor further specified), were used in this study (USDA water texture classification; p. 20; Figures 141-152, pp. 220-231 of MRID 50290803). The pH values were based on 1:1 water:water ratio results.

3 In the ILV, the Loam water (M963; CSR-7909-001; 48% sand 40% silt 12% clay, pH 5.9, 6.0% organic carbon) obtained from the sponsor was used in this study (USDA water texture classification; p. 14; Figure 5, p. 45 of MRID 50290803). The pH value was based on 1:1 water:water ratio result. The water source was not further specified.

I. Principle of the Method

For fluroxypyr-MHE and fluroxypyr acid:

Samples (20 mL) of water were transferred to 45-mL glass vials and fortified, as necessary, at the LOD, LOQ, 10×LOQ, and 100×LOQ (pp. 21.1R1, 22-23 of DAS# 081042**;** Appendix A, pp. 87-89 of MRID 50290803). The samples were acidified with 200 µL of concentrated formic acid solution. The solution was extracted with 5 mL of ethyl acetate and *ca*. 12 g NaCl by shaking for 10 minutes at *ca*. 180 excursions/minute on a flat-bed shaker. After centrifugation for 5 minutes at *ca*. 2000 rpm, the ethyl acetate layer was removed and transferred to a graduated Nalgene tube. The extraction was repeated with 5 mL of ethyl acetate. The combined extracts were mixed with 1.0 mL of methanol:water (50:50, v:v) solution containing 0.1% acetic acid. After pulse vortexing for about 10 seconds, the sample was concentrated to *ca*. 0.9 mL using a TurboVap evaporator set at 35-40 °C and a nitrogen pressure of 10-15 psi. The study author noted that this was a critical step and the sample should not be allowed to evaporate to dryness. The volume of the residue was adjusted to 2.0 mL using methanol:water (50:50, v:v) solution containing 0.1% acetic acid. After pulse vortexing for about 10 seconds, the sample was transferred to a 96-deep well plate for analysis by LC/MS/MS. Further dilution with using methanol:water (50:50, v:v) solution containing 0.1% acetic acid was prescribed for samples which had responses exceeding 80% of the highest calibration standard.

For fluroxypyr-DCP and fluroxypyr-MP:

Samples (60.0 mL) of water were transferred to 125-mL glass jars and fortified, as necessary, at the LOD, LOQ, 10×LOQ, and 100×LOQ (pp. 23-26 of DAS# 081042; Appendix A, pp. 89-92 of MRID 50290803). The samples were mixed with 6.0 mL of pH 7.00 buffer solution via hand shaking. A Phenomenex Strata-X solid phase extraction (SPE) cartridge (200 mg, 6 mL) was prepared by conditioning with 6 mL of methanol followed by 6 mL of pH 7.00 buffer solution. After the cartridge was dried under vacuum for 10 seconds, the extract mixture was applied to the column and pulled through at *ca*. 3-5 mL/minute. The sample vial was rinsed with 2 mL of pH 7.00 buffer solution and transferred to the SPE column. All eluates were discarded, the cartridge was dried under vacuum for about 20 minutes. The analytes were eluted with 2x2 mL of acetonitrile with 10 seconds of full vacuum between solvent additions. The eluate was collected into Nalgene tubes containing 1.0 mL of methanol:water (50:50, v:v) solution containing 0.1% acetic acid. The sample was concentrated to *ca*. 0.9 mL using a TurboVap evaporator set at 35-40 °C and a nitrogen pressure of 10-15 psi. The study author noted that this was a critical step and the sample should not be allowed to evaporate to dryness. The volume of the residue was adjusted to 1.5 mL using methanol:water (50:50, v:v) solution containing 0.1% acetic acid. After pulse vortexing for about 10 seconds, the sample was transferred to a 96-deep well plate for analysis by LC/MS/MS. Further dilution with using methanol:water (50:50, v:v) solution containing 0.1% acetic acid was prescribed for samples which had responses exceeding 80% of the highest calibration standard.

LC/MS/MS Analysis:

Samples were analyzed for analytes by Agilent 1100 HPLC (Zorbax SB-C8 column, 4.6 mm x 75 mm, 3.5 µm column; column temperature ambient) using a mobile phase of (A) methanol with 0.1% acetic acid and (B) water with 0.1% acetic acid [for fluroxypyr-MHE and fluroxypyr acid: percent A:B at 0.0-2.0 min. 60:40, 10.0-12.0 min. 100:0, 14.0-16.0 min. 60:40; for fluroxypyr-MP and fluroxypyr-DCP: percent A:B at 0.0-2.0 min. 40:60, 10.0 min. 100:0, 12.0-14.0 min. 40:60] with MDS/Sciex API 4000 MS using multiple reaction monitoring (MRM; pp. 16-19 of DAS# 081042; Appendix A, pp. 82-85 of MRID 50290803). For fluroxypyr-MHE and fluroxypyr acid, injection volume was 30 µL, and mass spectrometry was conducted with ESI (electrospray ionization; temperature 500°C) detection in negative polarity. For fluroxypyr-MP and fluroxypyr-DCP, injection volume was 20 µL, and mass spectrometry was conducted with APCI (atmospheric pressure chemical ionization; temperature 550°C) detection in positive polarity. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 365.1→194.1 and *m/z* 367.0→196.0 for fluroxypyr-MHE; *m/z* 252.9→232.9 and *m/z* 255.0→196.8 for fluroxypyr acid; *m/z* 199.0→181.0 and *m/z* 199.0→154.1 for fluroxypyr-DCP; and *m/z* 210.9→113.2 and *m/z* 210.9→196.1 for fluroxypyr-MP. Expected retention times were ca. 8.25, 2.03, 4.02, and 6.75 minutes for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, respectively (Figures 9-32, pp. 62R2-85R2).

ILV:

In the ILV, the ECM was performed as written, except for insignificant modifications to the analytical instrumentation and the fact that three or four 2.0 mL aliquots of acetonitrile were used

to elude the analytes from the Strata-X SPE, instead of two aliquots (pp. 17-20, 22 of MRID 50290803). An Agilent 1100 Infinity HPLC coupled with MDS/Sciex API 5000 MS using MRM was used for all analyses. The analytical parameters were the same except for some modifications of temperatures and injection volumes. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 365.2→194.1 and *m/z* 367.2→196.3 for fluroxypyr-MHE; *m/z* 253.2→232.9 and *m/z* 255.1→197.0 for fluroxypyr acid; *m/z* 199.14→181.00 and *m/z* 199.14→154.00 for fluroxypyr-DCP; and *m/z* 211.17→113.00 and *m/z* 211.17→195.90 for fluroxypyr-MP. Observed retention times were *ca*. 9.35-9.40, 2.06-2.07, 4.00-4.02, and 3.96-3.97 minutes for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-MP and fluroxypyr-DCP, respectively (Figures 5-44, pp. 35-74). No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) in the ECM and ILV was reported as 0.05 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP (pp. 2, 11, 30.1R2, 31R2; Tables 34-37, p. 53R2 of DAS# 081042; pp. 11, 23; Appendix A, pp. 77, 97 of MRID 50290803). The Limit of Detection (LOD) in the ECM was reported as 0.015 µg/L for all four analytes; the LOD was not reported in the ILV. In the ECM, the LOQ were calculated as 0.0233-0.0461 µg/L, 0.0310-0.0533 µg/L, 0.0403-0.0611 µg/L, and 0.0536-0.0715 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, respectively. The LOD were calculated as 0.0070-0.0138 µg/L, 0.0093-0.0166 µg/L, 0.0121-0.0183 µg/L, and 0.0161-0.0215 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, respectively.

II. Recovery Findings

ECM (DAS# 081042): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP in three water matrices at the fortification levels of 0.05 µg/L (LOQ), 0.5 µg/L (10×LOQ), and 5.0 µg/L (100×LOQ), except for fluroxypyr-MHE at the LOQ in drinking water (mean 63-64%) and surface water (mean 60-66%) and fluroxypyr-MHE at 10×LOQ in surface water [mean 50-52%, RSD 23.2% (Q); Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2; DER Attachment 2]. The number of samples prepared at 10×LOQ was insufficient for all analytes/matrices/ions (n = 3). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Recovery results were corrected when residues were quantified in the controls; residues were quantified in the control samples for fluroxypyr-MHE and fluroxypyr-MP in ground water (Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2; Figures 33-36, pp. 86R2-89R2). Recoveries for the LOD samples (0.015 µg/L) were reviewer-calculated; mean recovery, s.d. and RSDs could not be determined since n = 1 or 2. The LOD recoveries were 21-98% for fluroxypyr-MHE, 71-143% for fluroxypyr acid, 65-111% for fluroxypyr-DCP, and 69-109% for fluroxypyr-MP (matrices/ions combined); generally, the LOD recoveries of fluroxypyr-MHE and fluroxypyr acid were more variable and more outside the acceptable recovery range than the LOD recoveries of fluroxypyr-DCP and fluroxypyr-MP (Tables 2-13, pp. 37R2-48R2 and Tables 18-29, pp. 52.1R2-52.12R2; DER Attachment 2). The drinking, surface, and ground water matrices were not characterized; the locations of the water sources were not reported.

ILV (MRID 50290803): Mean recoveries and RSDs were within guideline requirements for analysis of fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP in three water matrices at the fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; Tables 1-6, pp. 25-30; DER Attachment 2). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Surface water (CSR No. 4626-001; pH 7.6, total hardness 41.0 mg $CaCO_3$/L, 1.45 mg/L total organic carbon, 1.47 mg/L dissolved organic carbon) obtained from the River Meon, Meonstoke, Hampshire, ground water (CSR No. 4626-002; pH 7.5, total hardness 201.2 mg $CaCO_3$/L, 0.722 mg/L total organic carbon, 0.607 mg/L dissolved organic carbon) obtained from a well near Henley-on-Thames, and drinking water (CSR No. 4626-003; pH 7.4, total hardness 307.3 mg $CaCO_3$/L, 3.25 mg/L total organic carbon, 2.87 mg/L dissolved organic carbon) obtained from a "drinking water" tap at the Test Facility (CEMAS) were used in this study (p. 12). The method for all four analytes was validated in the first trial with the use of three or four 2.0 mL aliquots of acetonitrile to elude the analytes from the Strata-X SPE, instead of two aliquots, as well as insignificant modifications to the analytical instrumentation (pp. 17-22).

Table 2. Initial Validation Method Recoveries for Fluroxypyr-MHE and Its Metabolites, Fluroxypyr acid, Fluroxypyr-DCP, and Fluroxypyr-MP, in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	**1**	**61**	--	--	--
	0.05 (LOQ)	5	**55**-78	**63**	9.2	14.5
	0.5	**3**	72-77	74	2.2	3.0
	5.0	5	71-81	76	4.3	5.7
Fluroxypyr acid	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	88-102	94	6.2	6.6
	0.5	**3**	88-92	90	2.3	2.5
	5.0	5	90-97	94	2.3	2.5
Fluroxypyr-DCP	0.015 (LOD)	**2**	**65**, 83	--	--	--
	0.05 (LOQ)	5	87-115	101	12.1	12.1
	0.5	**3**	98-100	99	1.2	1.2
	5.0	5	94-104	97	4.4	4.6
Fluroxypyr-MP	0.015 (LOD)	**2**	**69**, 99	--	--	--
	0.05 (LOQ)	5	95-123	104	11.3	10.9
	0.5	**3**	94-103	99	4.9	4.9
	5.0	5	84-92	89	3.1	3.4
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	**1**	**57**	--	--	--
	0.05 (LOQ)	5	**59**-75	**64**	6.6	10.4
	0.5	**3**	70-75	72	2.1	2.9
	5.0	5	**66**-84	76	7.3	9.6
Fluroxypyr acid	0.015 (LOD)	**1**	101	--	--	--
	0.05 (LOQ)	5	73-89	80	6.2	7.8
	0.5	**3**	88-92	89	2.3	2.6
	5.0	5	91-95	93	1.7	1.8
Fluroxypyr-DCP	0.015 (LOD)	**2**	103, 111	--	--	--
	0.05 (LOQ)	5	104-120	112	6.9	6.2
	0.5	**3**	99-100	100	0.6	0.6
	5.0	5	92-96	94	1.6	1.7
Fluroxypyr-MP	0.015 (LOD)	**2**	99, 103	--	--	--
	0.05 (LOQ)	5	95-126	109	12.6	11.5
	0.5	**3**	104-106	105	1.0	1.0
	5.0	5	88-100	95	5.5	5.8
	Groundwater[4]					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	**2**	**62**, 73	--	--	--
	0.05 (LOQ)	5	**60**-86	74	9.2	12.5
	0.5	**3**	71-88	79	8.5	10.7
	5.0	5	80-87	83	3.6	4.3
Fluroxypyr acid	0.015 (LOD)	**2**	**122**, **134**	--	--	--
	0.05 (LOQ)	5	93-109	99	6.5	6.5

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	3	90-101	95	5.1	5.4
	5.0	5	91-102	96	4.8	5.1
Fluroxypyr-DCP	0.015 (LOD)	1	104	--	--	--
	0.05 (LOQ)	5	92-112	103	8.1	7.8
	0.5	3	96-100	98	2.3	2.3
	5.0	5	97-102	99	2.2	2.2
Fluroxypyr-MP	0.015 (LOD)	1	86	--	--	--
	0.05 (LOQ)	5	75-107	91	14.3	15.8
	0.5	3	92-101	96	4.6	4.8
	5.0	5	91-96	94	2.0	2.1
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	2	80, 98	--	--	--
	0.05 (LOQ)	5	70-96	81	11.0	13.6
	0.5	3	80-85	82	2.6	3.2
	5.0	5	75-91	84	8.1	9.7
Fluroxypyr acid	0.015 (LOD)	2	139, 143	--	--	--
	0.05 (LOQ)	5	95-110	105	6.0	5.7
	0.5	3	95-100	97	2.6	2.7
	5.0	5	92-102	97	4.4	4.6
Fluroxypyr-DCP	0.015 (LOD)	1	105	--	--	--
	0.05 (LOQ)	5	90-108	98	7.0	7.2
	0.5	3	90-99	94	4.5	4.8
	5.0	5	90-103	96	5.0	5.2
Fluroxypyr-MP	0.015 (LOD)	1	109	--	--	--
	0.05 (LOQ)	5	89-98	94	3.9	4.2
	0.5	3	98-103	101	2.5	2.5
	5.0	5	96-100	98	1.7	1.7
	Surface Water					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	1	21	--	--	--
	0.05 (LOQ)	5	61-73	66	4.7	7.1
	0.5	3	41-65	52	12.1	23.2
	5.0	5	62-81	74	7.2	9.8
Fluroxypyr acid	0.015 (LOD)	1	90	--	--	--
	0.05 (LOQ)	5	96-122	111	11.1	9.9
	0.5	3	97-114	107	8.6	8.1
	5.0	5	88-97	92	3.7	4.0
Fluroxypyr-DCP	0.015 (LOD)	2	67, 109	--	--	--
	0.05 (LOQ)	5	92-114	100	8.2	8.2
	0.5	3	89-101	95	6.0	6.4
	5.0	5	92-101	96	3.9	4.0
Fluroxypyr-MP	0.015 (LOD)	2	82, 97	--	--	--
	0.05 (LOQ)	5	85-112	102	10.7	10.5
	0.5	3	88-99	93	5.6	6.0
	5.0	5	82-100	92	7.1	7.7

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.015 (LOD)	**1**	**47**	--	--	--
	0.05 (LOQ)	5	**47**-74	**60**	11.4	19.1
	0.5	**3**	**42**-**60**	**50**	9.1	18.0
	5.0	5	**65**-83	75	7.8	10.4
Fluroxypyr acid	0.015 (LOD)	**1**	71	--	--	--
	0.05 (LOQ)	5	98-129	112	13.2	11.8
	0.5	**3**	93-114	105	10.8	10.3
	5.0	5	89-96	93	3.3	3.6
Fluroxypyr-DCP	0.015 (LOD)	**2**	**67**, 93	--	--	--
	0.05 (LOQ)	5	75-115	101	15.6	15.4
	0.5	**3**	92-101	97	4.5	4.7
	5.0	5	85-96	90	4.1	4.6
Fluroxypyr-MP	0.015 (LOD)	**2**	80, 92	--	--	--
	0.05 (LOQ)	5	92-118	106	11.7	11.0
	0.5	**3**	93-102	98	4.6	4.7
	5.0	5	90-101	97	4.0	4.2

Data (recovery results were corrected when residues were quantified in the controls, Figures 33-36, pp. 86R2-89R2) were obtained from Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2 (LOQ, 10×LOQ and 100×LOQ results); and Tables 2-13, pp. 37R2-48R2 and Tables 18-29, pp. 52.1R2-52.12R2 (LOD results) of DAS# 081042 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 365.1→194.1 and *m/z* 367.0→196.0 for fluroxypyr-MHE; *m/z* 252.9→232.9 and *m/z* 255.0→196.8 for fluroxypyr acid; *m/z* 199.0→181.0 and *m/z* 199.0→154.1 for fluroxypyr-DCP; and *m/z* 210.9→113.2 and *m/z* 210.9→196.1 for fluroxypyr-MP.

2 The drinking, surface, and ground water matrices were not characterized; the locations of the waters were not reported.

3 Recoveries for the 0.015 µg/L samples (LOD) were reviewer-calculated based on data from Tables 2-16, pp. 37R2-48R2 and Tables 18-29, pp. 52.1R2-52.12R2 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 1 or 2.

4 Recovery results were corrected for residues quantified in the controls of fluroxypyr-MHE and fluroxypyr-MP (Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2; Figures 33-36, pp. 86R2-89R2).

Table 3. Independent Validation Method Recoveries for Fluroxypyr-MHE and Its Metabolites, Fluroxypyr acid, Fluroxypyr-DCP, and Fluroxypyr-MP, in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	87-98	93	5	5.0
	0.5	5	92-101	98	4	3.7
Fluroxypyr acid	0.05 (LOQ)	5	80-90	85	4	4.4
	0.5	5	80-88	83	3	3.5
Fluroxypyr-DCP	0.05 (LOQ)	5	**62**-100	86	15	17.4
	0.5	5	**67**-97	89	13	14.2
Fluroxypyr-MP	0.05 (LOQ)	5	84-87	86	2	1.6
	0.5	5	82-95	89	5	5.4
	Confirmatory ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	87-102	95	6	6.2
	0.5	5	92-103	96	5	4.8
Fluroxypyr acid	0.05 (LOQ)	5	90-99	94	4	4.4
	0.5	5	78-87	83	4	4.6
Fluroxypyr-DCP	0.05 (LOQ)	5	**61**-100	88	16	17.6
	0.5	5	**66**-98	88	13	14.6
Fluroxypyr-MP	0.05 (LOQ)	5	76-83	79	3	3.1
	0.5	5	84-91	89	3	4.0
	Ground Water					
	Quantitation ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	81-96	92	7	7.0
	0.5	5	89-99	94	5	5.0
Fluroxypyr acid	0.05 (LOQ)	5	80-97	86	6	7.5
	0.5	5	83-92	87	4	4.6
Fluroxypyr-DCP	0.05 (LOQ)	5	86-99	90	5	5.7
	0.5	5	88-96	92	3	3.3
Fluroxypyr-MP	0.05 (LOQ)	5	83-86	83	2	2.7
	0.5	5	79-90	85	4	5.0
	Confirmatory ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	84-100	94	7	6.9
	0.5	5	86-96	91	4	4.4
Fluroxypyr acid	0.05 (LOQ)	5	80-88	83	3	4.2
	0.5	5	80-90	84	4	5.1
Fluroxypyr-DCP	0.05 (LOQ)	5	92-97	94	2	2.5
	0.5	5	85-96	92	4	4.5
Fluroxypyr-MP	0.05 (LOQ)	5	82-87	84	2	2.5
	0.5	5	77-86	84	4	5.0
	Drinking Water					
	Quantitation ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	92-96	93	2	2.1
	0.5	5	89-100	94	5	5.9
Fluroxypyr acid	0.05 (LOQ)	5	82-91	89	4	4.3
	0.5	5	83-89	87	2	2.8

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Fluroxypyr-DCP	0.05 (LOQ)	5	94-106	99	5	5.6
	0.5	5	79-89	84	4	4.9
Fluroxypyr-MP	0.05 (LOQ)	5	79-103	90	9	10.2
	0.5	5	75-89	80	6	6.9
	Confirmatory ion					
Fluroxypyr-MHE	0.05 (LOQ)	5	94-99	97	2	2.1
	0.5	5	93-103	98	4	4.6
Fluroxypyr acid	0.05 (LOQ)	5	86-96	89	4	4.6
	0.5	5	85-90	88	2	2.6
Fluroxypyr-DCP	0.05 (LOQ)	5	92-111	101	9	8.8
	0.5	5	80-101	89	8	8.5
Fluroxypyr-MP	0.05 (LOQ)	5	83-107	90	10	10.8
	0.5	5	73-86	79	5	6.2

Data (uncorrected recovery results, pp. 14-17) were obtained from Tables 1-6, pp. 25-30 of MRID 50290803 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 365.2→194.1 and *m/z* 367.2→196.3 for fluroxypyr-MHE; *m/z* 253.2→232.9 and *m/z* 255.1→197.0 for fluroxypyr acid; *m/z* 199.14→181.00 and *m/z* 199.14→154.00 for fluroxypyr-DCP; and *m/z* 211.17→113.00 and *m/z* 211.17→195.90 for fluroxypyr-MP.

2 Surface water (CSR No. 4626-001; pH 7.6, total hardness 41.0 mg $CaCO_3$/L, 1.45 mg/L total organic carbon, 1.47 mg/L dissolved organic carbon) obtained from the River Meon, Meonstoke, Hampshire, ground water (CSR No. 4626-002; pH 7.5, total hardness 201.2 mg $CaCO_3$/L, 0.722 mg/L total organic carbon, 0.607 mg/L dissolved organic carbon) obtained from a well near Henley-on-Thames, and drinking water (CSR No. 4626-003; pH 7.4, total hardness 307.3 mg $CaCO_3$/L, 3.25 mg/L total organic carbon, 2.87 mg/L dissolved organic carbon) obtained from a "drinking water" tap at the Test Facility (CEMAS) were used in this study (p. 12).

3 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

III. Method Characteristics

The LOQ in the ECM and ILV was reported as 0.05 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP (pp. 2, 11, 30.1R2, 31R2; Tables 34-37, p. 53R2 of DAS# 081042; pp. 11, 23; Appendix A, pp. 77, 97 of MRID 50290803). The LOD in the ECM was reported as 0.015 µg/L for all four analytes; the LOD was not reported in the ILV. Following the method of Keith, L. H., *et al*. (see section V. References below), the LOD and LOQ for determination of fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP in water were calculated in the ECM using the standard deviation from the LOQ recovery results, which corresponded to 0.05 µg/g recovery results for all analytes. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. In the ECM, the LOQ were calculated as 0.0233-0.0461 µg/L, 0.0310-0.0533 µg/L, 0.0403-0.0611 µg/L, and 0.0536-0.0715 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, respectively. The LOD were calculated as 0.0070-0.0138 µg/L, 0.0093-0.0166 µg/L, 0.0121-0.0183 µg/L, and 0.0161-0.0215 µg/L for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, respectively. The calculated LOQ and LOD values supported the Method LOQ and LOD values.

Table 4. Method Characteristics

Analyte		Fluroxypyr-MHE	Fluroxypyr acid	Fluroxypyr-DCP	Fluroxypyr-MP
Limit of Quantitation (LOQ)	ECM	0.05 µg/L			
	ECM Calculated	0.0233-0.0461 µg/L	0.0310-0.0533 µg/L	0.0403-0.0611 µg/L	0.0536-0.0715 µg/L
	ILV	0.05 µg/L			
Limit of Detection (LOD)	ECM	0.015 µg/L			
	ECM Calculated	0.0070-0.0138 µg/L	0.0093-0.0166 µg/L	0.0121-0.0183 µg/L	0.0161-0.0215 µg/L
	ILV	**Not reported**			
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 0.9989 (Q)	r^2 = 0.9994 (Q)	r^2 = 0.9996 (Q)	r^2 = 0.9981 (Q)
	ILV	r^2 = 0.9997 (Q) r^2 = 0.9994 (C)	r^2 = 0.9998 (Q) r^2 = 0.9987 (C)	r^2 = 1.0000 (Q) r^2 = 0.9999 (C)	
	Range	0.25-50 ng/mL		0.5-50 ng/mL	
Repeatable	ECM[2]	Yes at 100×LOQ in three uncharacterized water matrices. Yes at the LOQ in ground water; **no** at LOQ in drinking water (mean **63**-**64**%) and surface water (mean **60**-**66**%). Yes at 10×LOQ (n = **3**) in drinking and ground water; **no** at 10×LOQ in surface water [mean **50**-**52**%, RSD **23.2**% (Q)].	Yes at the LOQ, 10×LOQ (n = **3**), and 100×LOQ in three uncharacterized water matrices.		
	ILV[3,4]	Yes at the LOQ and 10×LOQ in three characterized water matrices.			
Reproducible		Yes at LOQ and 10×LOQ, based on ILV results; **however**, the ECM should be updated with the ILV modifications.	Yes at the LOQ and 10×LOQ.		
Specific	ECM	Representative 10×LOQ and LOD chromatograms were **not** presented.			
		No, matrix interferences were *ca*. 11-**39**% (Q) and *ca*. 10-**30**% (C) of the LOQ (based on peak areas).[5]	Yes, no matrix interferences were observed; minor baseline noise which interfered with peak attenuation and integration at the LOQ was observed in some matrices.	Yes, matrix interferences were *ca*. 5-10% (Q) and *ca*. 10-12% (C) of the LOQ (based on peak areas).	

Analyte		Fluroxypyr-MHE	Fluroxypyr acid	Fluroxypyr-DCP	Fluroxypyr-MP
	ILV	Yes, matrix interferences were *ca*. 3-4% of the LOQ (based on peak areas).	Yes, no matrix interferences were observed; however, some baseline noise which interfered with peak attenuation and integration at the LOQ was observed.		Yes, matrix interferences were *ca*. 3% (Q) of the LOQ (based on residues quantified). Some peak tailing was observed.

Data were obtained from pp. 2, 11, 30.1R2, 31R2; Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2 (LOQ, 10×LOQ and 100×LOQ results); Tables 2-13, pp. 37R2-48R2 and Tables 18-29, pp. 52.1R2-52.12R2 (LOD results); Tables 34-37, p. 53R2 (calculated LOQ/LOD); Figures 5-8, pp. 58R2-61R2 (calibration curves); Figures 9-32, pp. 62R2-85R2 (chromatograms) of DAS# 081042; pp. 11, 23; Tables 1-6, pp. 25-30 (recovery data); Figures 1-4, pp. 31-34 (calibration curves); Figures 5-44, pp. 35-74 (chromatograms) of MRID 50290803. Q = Quantitation ion transition; C = Confirmation ion transition. Results are for both Q and C, unless specified otherwise.

1 Only quantitation ion transition calibration curves were reported and provided (p. 30R2).

2 In the ECM, the drinking, surface, and ground water matrices were not characterized; the locations of the waters were not reported.

3 In the ILV, surface water (CSR No. 4626-001; pH 7.6, total hardness 41.0 mg $CaCO_3$/L, 1.45 mg/L total organic carbon, 1.47 mg/L dissolved organic carbon) obtained from the River Meon, Meonstoke, Hampshire, ground water (CSR No. 4626-002; pH 7.5, total hardness 201.2 mg $CaCO_3$/L, 0.722 mg/L total organic carbon, 0.607 mg/L dissolved organic carbon) obtained from a well near Henley-on-Thames, and drinking water (CSR No. 4626-003; pH 7.4, total hardness 307.3 mg $CaCO_3$/L, 3.25 mg/L total organic carbon, 2.87 mg/L dissolved organic carbon) obtained from a "drinking water" tap at the Test Facility (CEMAS) were used in this study (p. 12).

4 In the ILV, the method for all four analytes was validated in the first trial with the use of three or four 2.0 mL aliquots of acetonitrile to elude the analytes from the Strata-X SPE, instead of two aliquots, and insignificant modifications to the analytical instrumentation (pp. 17-22).

5 Based on Figures 9-11, pp. 62R2-64R2 (Q) and Figures 21-23, pp. 74R2-76R2 (C) of DAS# 081042.

IV. Method Deficiencies and Reviewer's Comments

1. For the application of the method to fluroxypyr-MHE, an updated ECM should be submitted to include the ILV modifications of the use of three or four 2.0 mL aliquots of acetonitrile to elude the analytes from the Strata-X SPE, instead of two aliquots (pp. 17-22 of MRID 50290803). The repeatability and specificity of the method was not supported for fluroxypyr-MHE in the three water matrices by the ECM performance data or representative chromatograms [Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2; Figures 9-11, pp. 62R2-64R2 (Q) and Figures 21-23, pp. 74R2-76R2 (C) of DAS# 081042]. The reviewer assumed that the ILV modifications would improve the ECM recovery results, since the unsatisfactory ECM performance data was <70%.

2. The following deficiencies were noted in the **ECM**:

   Performance data was unsatisfactory for fluroxypyr-MHE at the LOQ in drinking water (mean 63-64%) and surface water (mean 60-66%) and fluroxypyr-MHE at 10×LOQ in surface water [mean 50-52%, RSD 23.2% (Q); Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2 of DAS# 081042). OCSPP guidelines state that mean recoveries and relative standard deviations (RSDs) should be 70-120% and ≤20%, respectively.

   The number of samples prepared at 10×LOQ was insufficient for all analytes/matrices/ions (n = 3; Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2 of DAS# 081042). OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

   The specificity of the method for fluroxypyr-MHE was not supported by ECM representative chromatograms due to significant matrix interferences [*ca*. 11-39% (Q) and *ca*. 10-30% (C) of the LOQ (based on peak areas); Figures 9-11, pp. 62R2-64R2 (Q) and Figures 21-23, pp. 74R2-76R2 (C) of DAS# 081042].

   Representative 10×LOQ and LOD chromatograms were not provided. Representative chromatograms from all matrices and fortifications should be provided for review.

   The drinking, surface, and ground water matrices were not characterized; the locations of the waters were not reported.

   Recovery results of fluroxypyr-MHE and fluroxypyr-MP were corrected for residues quantified in the controls (Tables 14-17, pp. 49R2-52R2 and Tables 30-33, pp. 52.13R2-52.16R2; Figures 33-36, pp. 86R2-89R2 of DAS# 081042).

   The calibration curves and correlation coefficients of the confirmation analysis were not reported. The reviewer noted that a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

3. In the ECM, the fortification and calibration solutions were found to be stable up to 99 and 20 days, respectively, of refrigerated storage (pp. 28-29, 30R2 of DAS# 081042). The final sample extracts were found to be stable up to 5 days at *ca*. 4°C (p. 25; Tables 40-43, pp. 72-75).

4. The ILV reported that no communications between the Study Director and the method developers was required (p. 22 of MRID 50290803).

5. It was reported for the ILV that one sample set of 12 samples and a reagent blank required *ca*. 8 person-hours (fluroxypyr-MHE and fluroxypyr acid) or *ca*. 6 person-hours (fluroxypyr-DCP and fluroxypyr-MP) to prepare with LC/MS/MS performed unattended overnight (p. 22 of MRID 50290803). Evaluation of the LC/MS/MS results was completed the following day in *ca*. 2 hours, so a sample set was completely processed and evaluated in *ca*. 1.5 working days.

6. A list of the amendment changes was reported in the ECM (p. 4.1R2 of DAS# 081042).

7. The reviewer noted that the original DAS# 081042 study report was written by D.D. Shackelford (Appendix A, p. 76 of MRID 50290803).

V. References

Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 35R2 of DAS# 081042; Appendix A, p. 101 of MRID 50290803).

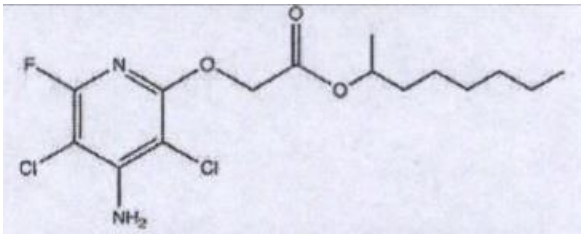
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

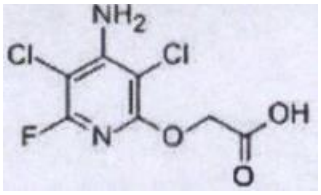
Fluroxypyr-MHE (Fluroxypyr 1-MHE; Fluroxypyr 1-meptyl)

IUPAC Name: ((4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid 1-methylheptyl ester
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



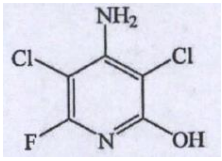
Fluroxypyr acid

IUPAC Name: ((4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



Fluroxypyr-DCP (Fluroxypyr-2-pyridinol; X061784)

IUPAC Name: 4-Amino-3,5-dichloro-6-fluoro-2-pyridinol
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



Fluroxypyr-MP (DMP fluroxypyr metabolite; X61420)

IUPAC Name: 4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found