Analytical method for fluazifop-p-butyl and its acid degradate (R156172) in soil

Reports:	ECM: EPA MRID No.: 49592401. Huang, S.-B. 2015. Fluazifop-P-Butyl: Fluazifop-P-Butyl – Analytical Method for Enantiomeric Ratio and Residue Determination of Fluazifop-P-Butyl (R154875; PP5) and its Acid Degradate (R156172) in Soil – Analytical Method. Report No.: GRM044.05A. Task No.: TK0062679. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 106 pages. Final report issued March 12, 2015. ILV: EPA MRID No. 49800201. Guo, D. 2015. Fluazifop-P-Butyl: Independent Laboratory Validation of Amended Analytical Method (GRM044.05A) for Enantiomeric Ratio and Residue Determination of Fluazifop-P-Butyl (R154875; PP5) and its Acid Degradate (R156172) in Soil – Final ILV Report. Report No.: PASC-REP-0656. PASC Project No.: 141-1187. Task No.: TK0278222. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 247 pages. Final report issued December 11, 2015.
Document No.:	MRIDs 49592401 & 49800201
Guideline:	850.6100
Statements:	ECM: The study was not conducted in compliance with USEPA FIFRA or OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49592401). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). A certification of authenticity and Quality Assurance statement were not included. A signed summary of revisions to previous method version was included (p. 4). ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 49800201). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not included.
Classification:	This analytical method is classified as **Unacceptable**. In the ECM, the number of samples was insufficient for all analyses, and no representative chromatograms were provided for the test matrix. In the ILV, some minor baseline noise and non-uniform integration was noted in the representative chromatograms for the S enantiomer for fluazifop at the LOQ.
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Final EPA Reviewer:	Richard Shamblen, Biologist Signature: RICHARD SHAMBLEN Digitally signed by RICHARD SHAMBLEN Date: 2019.07.02 16:20:18 -04'00' Date: June 26, 2019
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist Signature: Lisa Muto Date: 1/10/17 Kathleen Ferguson, Ph.D., Environmental Scientist Signature: Kathleen P. Ferguson Date: 1/10/17

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

Page numbers cited in ILV MRID 49800201 refer to those reported in the lower right-hand corner of the document.

Executive Summary

This analytical method, Syngenta Method GRM044.05A, is designed for the quantitative determination of the S and R enantiomers of fluazifop-p-butyl and fluazifop-p-acid (fluazifop) in soil at the LOQ of 1.0 µg/kg (1.0 ppb) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil. The analytical method provided an optional solid phase extraction (SPE) procedure for difficult soil matrices. The optional SPE procedure was not performed by the ILV; the ECM did not specify if the optional SPE extraction was performed. In the ECM, the method was validated using characterized sand soil; however, an insufficient number of samples was prepared for all analyses (n = 3). Additionally, no representative chromatograms were provided for the test matrix. Two ion transitions were reported in the method, but only the primary ion transitions were monitored. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS/MS. An updated ECM should be submitted with acceptable chromatographic support. The ILV validated the method for all four analytes using characterized clay loam and sandy loam soils after one trial with insignificant modifications to the analytical method. Two ion transitions were monitored in the ILV. For fluazifop, some minor baseline noise and non-uniform integration was noted for the S enantiomer at the LOQ.

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						
S enantiomer of Fluazifop-P-Butyl (R159618)	49592401[2,3]	49800201[4]		Soil	12/03/2015	Syngenta Crop Protection, LLC	LC/MS/MS	1.0 µg/kg; 0.001 mg/kg; 1.0 ppb
R enantiomer of Fluazifop-P-Butyl (R154875; PP5)								
S enantiomer of Fluazifop-P-Acid[1] (R159697)								
R enantiomer of Fluazifop-P-Acid[1] (R156172)								

1 Referred to as Fluazifop in DER.

2 In the ECM, sand soil (90% sand, 8% silt, 2% clay; pH 6.3 in 0.01M $CaCl_2$; 0.55% organic carbon) was characterized in Syngenta Study TK0015266 Final Report (Table 13), August 2, 2013 (Terrestrial field dissipation study; Table 1, p. 30 of MRID 49592401). USDA soil texture classification was not specified. The source was not specified.

3 ECM recovery data was obtained from Syngenta Study TK0015266, Analytical Phase Report Amendment 2 (November 26, 2014; Tables 2-3, pp. 31-32 of MRID 49592401).

4 In the ILV, clay loam soil (21% sand, 42% silt, 37% clay; pH 7.6 in 0.01M $CaCl_2$; % organic carbon not reported) was obtained from Underwood farm, 0-6" depth (Table 1, p. 33 of MRID 49800201). The sandy loam soil (55% sand, 28% silt, 17% clay; pH 7.3 in 0.01M $CaCl_2$; % organic carbon not reported) and obtained from Madera, California, 1-15-13, 0-6" depth. Both soils were characterized in Syngenta Study TK0002309 by Agvise Laboratories, Northwood, North Dakota; USDA soil texture classification was not specified. The sources were not further specified.

I. Principle of the Method

Soil samples (20 g) were weighed into 50-mL polypropylene centrifuge tubes and fortified, if necessary. After 5 minutes of equilibration of the fortification solution, the soil was extracted twice with acetonitrile:10 mM ammonium acetate buffer at pH 5 (50:50, v:v; 30 mL first extraction, 20 mL second extraction) by shaking at room temperature for 20 minutes (speed not specified); tubes should be placed in the horizontal position during shaking (pp. 16-17; Appendices 1-4, pp. 102-106 of MRID 49592401). After centrifugation at *ca*. 5000 rpm for *ca.* 10 minutes, the supernatant was transferred to a clean 50-mL centrifuge tube. The method noted that it was acceptable for the supernatant of some soils, especially those with a high clay content, may remain cloudy after centrifugation. The volume of the combined extracts was adjusted to 50 mL with 10 mM ammonium acetate buffer at pH 5. An aliquot (1 mL) was filtered with a 1-mL glass syringe containing a PTFE syringe membrane filter (13 mm; 0.2 µm). A 500-µL aliquot of the filtered extract was diluted with 500 µL of 10 mM ammonium acetate buffer at pH 5 (2x dilution) prior to analysis via LC/MS/MS. Further dilution with acetonitrile:10 mM ammonium

acetate buffer at pH 5 (50:50, v:v) may be necessary when sample contain more than 1000 ppb of residues or interferences are detected.

An optional solid phase extraction (SPE) clean-up was provided in case insufficient instrument sensitivity or significant matrix effects interfered with results (p. 17 of MRID 49592401). The Waters Oasis® MAX SPE cartridge (150 mg, 6-mL) was preconditioned sequentially with methanol (3 mL x 2), acetonitrile (3 mL x 2), methanol (3 mL x 2), 0.01% NH_4OH in water (3 mL x 2) and water (3 mL x 2). An aliquot (5 mL) of the final combined extracts (from above) were diluted with 5.0 mL of 10 mM ammonium acetate buffer at pH 5 in a 15-mL polypropylene centrifuge tube. The diluted soil extract was applied to the column, portion-wise with a slight positive pressure or vacuum (flow less than 20 drops/minute). The polypropylene centrifuge tube was rinsed with 2.0 mL of 0.1% formic acid in methanol:ultra-pure water (20:80, v:v) and then ultra-pure water (3 mL x 2). Each of the rinsates were used to wash the SPE column. The analytes were collected with acidified (1% formic acid) acetonitrile:acetone (25:75, v:v; 2 mL x 3). The eluate was collected into a clean 15-mL polypropylene centrifuge tube. The eluate was evaporated to dryness under a gentle stream of nitrogen ro air at a bath temperature of ca. 40C. Acetonitrile (0.3 mL) was added to the centrifuge tube and vortexed. The final volume was adjusted to 1 mL with 10 mM ammonium acetate buffer at pH 5 or an appropriate volume of acetonitrile:10 mM ammonium acetate buffer at pH 5 (30:70, v:v). An aliquot of the final sample was transferred to an autosampler vial for analysis by LC/MS/MS. Further dilution with acetonitrile:10 mM ammonium acetate buffer at pH 5 (30:70, v:v) may be necessary.

Samples were analyzed for fluazifop-P-butyl (S enantiomer, R159618; R enantiomer, PP5) and fluazifop (S enantiomer, R159697; R enantiomer, R156172) by Surveyor Plus LC system (Chiralpak AS-RH, 150 x 4.6 mm, 5.0 µm column; column temperature 25°C) with a column filter (ColumnSaver) using a gradient mobile phase of (A) 0.1% formic acid in Optima grade water, (B) HPLC grade 2-propanol and (C) 0.1% formic acid in HPLC grade acetonitrile [time ratio A:B:C; 0.0-3.0 min. 50:5:45, 4.0-12.0 min. 35:5:60, 12.1-13.0 min. 50:5:45] coupled with a Thermo Electron TSQ Quantum Ultra mass spectrometer with HESI-II probe (300°C) in positive ion or negative ion mode (Multiple Reaction Monitoring mode, MRM; pp. 19-22). Analytes were identified with two transitions, primary and confirmation ion transitions. Positive mode was employed for fluazifop-P-butyl (R and S enantiomers) with transitions of 384.10→282.10 and 384.14→328.10. Negative mode was employed for fluazifop (R and S enantiomers) with transitions of 326.10→254.10 and 326.10→226.10. Injection volumes were 50 µL. Retention times were 10.7 minutes for fluazifop-P-butyl (R and S enantiomers), 5.8 minutes for fluazifop (S enantiomer) and 6.5 minutes for fluazifop (R enantiomer).

In the ILV, the ECM was performed as written, except that mobile phase B was added to solvents A and C at 5% and eliminated (pp. 23-26 of MRID 49800201). The SPE extraction was not performed. The analytical instrument was a Waters Acquity UPLC system coupled to an Applied Biosystems Sciex API 6500 triple quadrupole mass spectrometer. The mobile phase was adjusted to (A) 0.1% formic acid in water with 5% 2-propanol and (B) 0.1% formic acid in acetonitrile with 5% 2-propanol [time ratio A:B; 0.0-3.0 min. 52.6:47.4, 4.0-12.0 min. 36.8:63.2, 12.1-13.0 min. 52.6:47.4. Approximate retention times were *ca*. 9.82 minutes for fluazifop-P-butyl (S enantiomer), 10.31 minutes for fluazifop-P-butyl (R enantiomer), 5.70 minutes for fluazifop (S enantiomer) and 6.36 minutes for fluazifop (R enantiomer. The ILV study author

noted that the S enantiomer was not supplied by the sponsor, so the retention time of the S enantiomer was established using the racemic mixture (p. 19). Monitored transitions basically matched those reported in the ECM (p. 26). No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for fluazifop-P-butyl and fluazifop was reported as 1.0 µg/kg (1.0 ppb; 0.001 mg/kg) in the ECM and the ILV (pp. 11, 26 of MRID 49592401; pp. 23, 27 of MRID 49800201). The Limit of Detection (LOD) for the individual enantiomers was 0.05 ng/mL when using a 50 µL injection volume (2.5 pg injected on column) in the ECM. The LOD was not specifically reported in the ILV, but the lowest calibration standard was the 0.05 ng/mL individual enantiomer concentration level.

II. Recovery Findings

ECM (MRID 49592401): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of S and R enantiomers of fluazifop-P-butyl at fortification levels of 1.0 µg/kg (LOQ), 10 µg/kg (10x LOQ) and 100 µg/kg (100x LOQ) and S and R enantiomers of fluazifop at fortification levels of 1.0 µg/kg (LOQ), 10 µg/kg (10x LOQ) and 500 µg/kg (500x LOQ) in sand soil (p. 26; Tables 2-3, pp. 31-32; DER Attachment 2). ECM recovery data was obtained from Syngenta Study TK0015266, Analytical Phase Report Amendment 2 (November 26, 2014; terrestrial field dissipation study); it was not specified if the optional SPE extraction was performed in that study. The number of samples was insufficient for all analyses (n = 3). Two ion transitions were reported in the method, but only the primary ion transitions were monitored. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS/MS. Standard deviations were reviewer-calculated using the individual recovery values reported in the study report since standard deviations were not provided by the study author (DER Attachment 2). The means and RSDs for fluazifop (S) at 10 and 500 µg/kg were reviewer-calculated since the values provided in the study report were erroneous (for 10 µg/kg: mean 102% and RSD 13%; for 500 µg/kg: mean 107% and RSD 4.6%; Table 3, p. 32). The sand soil (90% sand, 8% silt, 2% clay; pH 6.3 in 0.01M $CaCl_2$; 0.55% organic carbon) was characterized in Syngenta Study TK0015266 Final Report (Table 13), August 2, 2013 (Table 1, p. 30). USDA soil texture classification was not specified. The source was not specified.

ILV (MRID 49800201): Mean recoveries and RSDs were within guideline requirements for analysis of S and R enantiomers of fluazifop-P-butyl and fluazifop in clay loam and sandy loam soils at fortification levels of 0.001 mg/kg (LOQ) and 0.01 mg/kg (10x LOQ; Tables 3-10, pp. 35-42). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The clay loam soil (21% sand, 42% silt, 37% clay; pH 7.6 in 0.01M $CaCl_2$; % organic carbon not reported) was obtained from Underwood farm, 0-6” depth (Table 1, p. 33). The sandy loam soil (55% sand, 28% silt, 17% clay; pH 7.3 in 0.01M $CaCl_2$; % organic carbon not reported) and obtained from Madera, California, 1-15-13, 0-6” depth. Both soils were characterized in Syngenta Study TK0002309 by Agvise Laboratories, Northwood, North Dakota; USDA soil texture classification was not specified. The sources were not further specified. The method was validated for all analytes in

both matrices after one trial with insignificant modifications to the analytical method (pp. 23-24, 27).

Table 2. Initial Validation Method Recoveries for Fluazifop-p-butyl and Fluazifop in Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[2]	Relative Standard Deviation (%)
	Sand Soil[3]					
	Primary Transition					
Fluazifop-p-butyl (R enantiomer)	1.0 (LOQ)	3	85-89	86	2	2.7
	10	3	71-85	76	8	10
	100	3	90-101	95	6	5.9
Fluazifop-p-butyl (S enantiomer)	1.0 (LOQ)	3	88-91	90	2	1.7
	10	3	71-85	76	8	11
	100	3	90-100	94	6	5.9
Fluazifop (R enantiomer)	1.0 (LOQ)	3	77-87	81	5	6.3
	10	3	87-105	93	10	11
	500	3	90-97	93	4	3.9
Fluazifop (S enantiomer)	1.0 (LOQ)	3	98-107	102	5	4.5
	10	3	94-117	104[4]	12	11[4]
	500	3	107-110	109[4]	2	1[4]
	Confirmatory Transition					
Fluazifop-p-butyl (R enantiomer)	1.0 (LOQ)	3	Not reported			
	10	3				
	100	3				
Fluazifop-p-butyl (S enantiomer)	1.0 (LOQ)	3				
	10	3				
	100	3				
Fluazifop (R enantiomer)	1.0 (LOQ)	3				
	10	3				
	500	3				
Fluazifop (S enantiomer)	1.0 (LOQ)	3				
	10	3				
	500	3				

Data (uncorrected recovery results, pp. 22-23) were obtained from Tables 2-3, pp. 31-32 of MRID 49592401 and DER Attachment 2.

1 The ECM recovery data was obtained from Syngenta Study TK0015266, Analytical Phase Report Amendment 2 (November 26, 2014; terrestrial field dissipation study); it was not specified if the optional SPE extraction was performed in that study (p. 26; Tables 2-3, pp. 31-32).

2 Standard deviations were reviewer-calculated using the individual recovery values reported in the study report since standard deviations were not provided by the study author (DER Attachment 2).

3 The sand soil (90% sand, 8% silt, 2% clay; pH 6.3 in 0.01M $CaCl_2$; 0.55% organic carbon) was characterized in Syngenta Study TK0015266 Final Report (Table 13), August 2, 2013 (Table 1, p. 30). USDA soil texture classification was not specified. The source was not specified.

4 The means and RSDs for fluazifop (S) at 10 and 500 µg/kg were reviewer-calculated since the values provided in the study report were erroneous (for 10 µg/kg: mean 102% and RSD 13%; for 500 µg/kg: mean 107% and RSD 4.6%; Table 3, p. 32).

Table 3. Independent Validation Method Recoveries for Fluazifop-p-butyl and Fluazifop in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Clay Loam Soil[1]					
	Primary Transition					
Fluazifop-p-butyl (S enantiomer)	0.001 (LOQ)	5	83-90	87	2	3
	0.01	5	81-88	84	3	3
Fluazifop-p-butyl (R enantiomer)	0.001 (LOQ)	5	84-92	87	3	4
	0.01	5	80-88	82	3	4
Fluazifop (S enantiomer)	0.001 (LOQ)	5	88-97	92	3	4
	0.01	5	85-90	87	2	2
Fluazifop (R enantiomer)	0.001 (LOQ)	5	93-98	95	2	2
	0.01	5	88-92	89	1	1
	Confirmatory Transition					
Fluazifop-p-butyl (S enantiomer)	0.001 (LOQ)	5	83-89	86	2	3
	0.01	5	81-88	83	3	4
Fluazifop-p-butyl (R enantiomer)	0.001 (LOQ)	5	84-93	87	3	4
	0.01	5	79-87	82	3	4
Fluazifop (S enantiomer)	0.001 (LOQ)	5	87-93	90	3	3
	0.01	5	84-89	86	2	2
Fluazifop (R enantiomer)	0.001 (LOQ)	5	97-106	101	4	4
	0.01	5	87-90	88	1	1
	Sandy Loam Soil[1]					
	Primary Transition					
Fluazifop-p-butyl (S enantiomer)	0.001 (LOQ)	5	98-101	100	1	1
	0.01	5	90-95	92	2	2
Fluazifop-p-butyl (R enantiomer)	0.001 (LOQ)	5	98-101	100	2	2
	0.01	5	92-97	94	2	2
Fluazifop (S enantiomer)	0.001 (LOQ)	5	93-100	97	3	3
	0.01	5	90-95	91	2	2
Fluazifop (R enantiomer)	0.001 (LOQ)	5	94-105	100	4	4
	0.01	5	90-95	92	2	2
	Confirmatory Transition					
Fluazifop-p-butyl (S enantiomer)	0.001 (LOQ)	5	99-101	100	1	1
	0.01	5	90-94	91	2	2
Fluazifop-p-butyl (R enantiomer)	0.001 (LOQ)	5	96-101	99	2	2
	0.01	5	92-97	94	2	2
Fluazifop (S enantiomer)	0.001 (LOQ)	5	90-103	97	4	5
	0.01	5	89-91	91	2	2
Fluazifop (R enantiomer)	0.001 (LOQ)	5	84-105	94	8	8
	0.01	5	87-93	90	2	3

Data (uncorrected recovery results, Appendix 1, pp. 142-143) were obtained from Tables 3-10, pp. 35-42 of MRID 49800201.

1 The clay loam soil (21% sand, 42% silt, 37% clay; pH 7.6 in 0.01M $CaCl_2$; % organic carbon not reported) was obtained from Underwood farm, 0-6" depth (Table 1, p. 33). The sandy loam soil (55% sand, 28% silt, 17% clay; pH 7.3 in 0.01M $CaCl_2$; % organic carbon not reported) and obtained from Madera, California, 1-15-13, 0-6" depth. Both soils were characterized in Syngenta Study TK0002309 by Agvise Laboratories, Northwood, North Dakota; USDA soil texture classification was not specified. The sources were not further specified.

III. Method Characteristics

The LOQ for fluazifop-P-butyl and fluazifop was reported as 1.0 µg/kg (1.0 ppb; 0.001 mg/kg) in the ECM and the ILV (pp. 11, 26 of MRID 49592401; pp. 23, 27 of MRID 49800201). In the ECM, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of 20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justification for the LOQ was provided in the ILV. The LOD for the individual enantiomers was 0.05 ng/mL when using a 50 µL injection volume (2.5 pg injected on column) in the ECM. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The ECM also stated that an estimate of the LOD can be taken as three times background noise. The LOD was not specifically reported in the ILV, but the lowest calibration standard was the 0.05 ng/mL individual enantiomer concentration level. No justification for the LOD was provided in the ILV.

Table 4. Method Characteristics

Analyte		**Fluazifop-p-butyl**	**Fluazifop**
Limit of Quantitation (LOQ)	ECM	1.0 µg/kg (0.001 mg/kg; 1.0 ppb)	
	ILV		
Limit of Detection (LOD)	ECM	0.05 ng/mL when using a 50 µL injection volume (2.5 pg injected on column)	
	ILV	Not reported	
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = 0.9985 (S,Q) r^2 = 0.9976 (S,C) r^2 = 0.9986 (R,Q) r^2 = 0.9976 (R,C)	r^2 = 0.9997 (S,Q) r^2 = 0.9994 (S,C) r^2 = 0.9996 (R,Q) r^2 = 0.9991 (R,C)
	ILV[1]	r^2 = 0.9974 (S,Q) r^2 = 0.9976 (S,C) r^2 = 0.9976 (R,Q) r^2 = 0.9982 (R,C)	r^2 = 0.9996 (S,Q) r^2 = 0.9996 (S,C) r^2 = 0.9996 (R,Q) r^2 = 0.9986 (R,C)
	Concentration Range	(0.05-10 µg/L)	
Repeatable	ECM[2,3,4]	**Insufficient** samples were prepared (n=**3**)	
		Yes at LOQ, 10×LOQ and 100×LOQ	Yes at LOQ, 10×LOQ and 500×LOQ
	ILV[5,6]	Yes at LOQ and 10×LOQ	
Reproducible		Yes at LOQ and 10×LOQ	
Specific	ECM	**Could not be determined**. Chromatograms were not provided for the test matrix.[7] Matrix interferences reported as <30% of the LOQ.	
	ILV	Yes, no matrix interferences were observed.	Yes, matrix interferences were <1% for the primary transition and <10% for the confirmatory transition. Some minor baseline noise and non-uniform integration was noted for the S enantiomer at the LOQ.[8]

Data were obtained from pp. 11, 26-27; Tables 2-3, pp. 31-32 (recovery data); Figures 8-18, pp. 46-87 (chromatograms); Figures 19-26, pp. 89-96 (calibration curves) of MRID 49592401; pp. 23, 27; Tables 3-10, pp. 35-42 (recovery data); Figures 9-134, pp. 48-110 (calibration curves and chromatograms) of MRID 49800201 and DER Attachment 2. S = S enantiomer; R = R enantiomer; Q = primary ion transition; C = confirmatory ion transition.

1 For the ILV, correlation coefficients for all analytes were reviewer-calculated from r values provided in the study report (Figures 25-26, p. 56; Figures 57-58, p. 72; Figures 89-90, p. 88; Figures 121-122, p. 104 of MRID 49800201; DER Attachment 2).

2 In the ECM, sand soil (90% sand, 8% silt, 2% clay; pH 6.3 in 0.01M $CaCl_2$; 0.55% organic carbon) was characterized in Syngenta Study TK0015266 Final Report (Table 13), August 2, 2013 (Table 1, p. 30 of MRID 49592401). USDA soil texture classification was not specified. The source was not specified.

3 In the ECM. two ion transitions were reported in the method, but only the primary ion transition was monitored. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS/MS.

4 ECM recovery data was obtained from Syngenta Study TK0015266, Analytical Phase Report Amendment 2 (November 26, 2014; Tables 2-3, pp. 31-32 of MRID 49592401).

5 In the ILV, clay loam soil (21% sand, 42% silt, 37% clay; pH 7.6 in 0.01M $CaCl_2$; % organic carbon not reported) was obtained from Underwood farm, 0-6" depth (Table 1, p. 33 of MRID 49800201). The sandy loam soil (55% sand, 28% silt, 17% clay; pH 7.3 in 0.01M $CaCl_2$; % organic carbon not reported) and obtained from Madera, California, 1-15-13, 0-6" depth. Both soils were characterized in Syngenta Study TK0002309 by Agvise Laboratories, Northwood, North Dakota; USDA soil texture classification was not specified. The sources were not further specified.

6 The ILV validated the method for all analytes in both matrices after one trial with insignificant modifications to the analytical method (pp. 23-24, 27 of MRID 49800201).

7 Chromatograms were only provided for loam and sandy loam soil matrices; neither of which was a test matrix.

8 Based on Figures 93-94, p. 90 and Figures 99-100, p. 93 of MRID 49800201.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV MRID 49800201 was performed based on an updated version of ECM MRID 49592401 entitled "Fluazifop-P-Butyl: Fluazifop-P-Butyl – Analytical Method for Enantiomeric Ratio and Residue Determination of Fluazifop-P-Butyl (R154875; PP5) and its Acid Degradate (R156172) in Soil – Amended Analytical Method" (June 25, 2015; Appendix 1, pp. 121-226 of MRID 49800201). The only change to the original method was the corrections of a typographical error in the title of several figures: S-Fluazifop-Butyl was changed to R-Fluazifop-Butyl (Appendix 1, p. 124, 131). The entire Amended ECM was provided in Appendix 1 of the ILV. This Amended ECM was reviewed and determined to be the same as MRID 49592401, except for the typographical error changes.

2. In the ECM, the number of samples was insufficient for all analyses (n = 3; Tables 2-3, pp. 31-32 of MRID 49592401). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.

3. In the ECM, chromatograms were not provided to confirm the specificity of the method (Figures 8-18, pp. 46-87 of MRID 49800201). Chromatograms were not provided for the test matrix, sand soil; chromatograms were only provided for loam and sandy loam soil matrices. The representative chromatograms were noted as from "Method Development" (p. 39). An updated ECM should be submitted with acceptable chromatographic support. Representative chromatograms from the test matrix should be provided for review of method specificity.

4. In the ILV, the reviewer noted that some minor baseline noise (<30% of the LOQ) and non-uniform integration was noted in the representative chromatograms for the S enantiomer of fluazifop at the LOQ (Figures 93-94, p. 90; Figures 99-100, p. 93 of MRID 49800201).

5. The reviewer noted that the sand soil was characterized in Table 1 which was titled "Charaterisation Data for Soil Samples Used in TK0015266 Method Trial" (Table 1, p. 30 of MRID 49592401). Syngenta TK0015266 was identified as a terrestrial field dissipation study (p. 26). Additionally, the reviewer noted that the study author stated that "the method recovery data obtained from TK0015266 [was] based on a multi point approach" (p. 26).

6. The estimations of LOQ and LOD in ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 ILV (pp. 11, 26 of MRID 49592401; pp. 23, 27 of MRID 49800201). In the ECM, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of 20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justification for the LOQ was provided in the ILV. The LOD for the individual enantiomers was 0.05 ng/mL when using a 50 µL injection volume (2.5 pg injected on column) in the ECM. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The ECM also stated that an estimate of the LOD can be taken as three times background noise. The LOD was not specifically reported in the ILV, but the lowest calibration standard was the 0.05 ng/mL individual enantiomer concentration level. No calculations were reported to justify the LOQ and LOD for the method.

7. Communications between the ILV and study monitor were summarized as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standard and control sample, and 3) acquisition of the revised method with changes to the test procedures (p. 30 of MRID 49800201). The list of email communications was provided in Appendix 6, pp. 241-247.

8. In the ECM and ILV, no significant matrix effects (>20%) were observed for soils (p. 27; Table 4, p. 33 of MRID 49592401; p. 30 of MRID 49800201). However, the soils for

which matrix effects were assessed in the ECM were reported as Loam and Sandy Loam, not the test soil, sand.

9. In the ECM, recovery of fluazifop-p-butyl and fluazifop in soil in acetonitrile:10 mM ammonium acetate buffer at pH 5 (30:70; v:v) was found to be acceptable (>80%) after 7 days of refrigerated storage (4°C; p. 27; Tables 5-6, p. 34 of MRID 49592401). However, the soils for which storage stability was assessed in the ECM were reported as Loam and Sandy Loam, not the test soil, sand.

10. The reviewer noted that the raw chromatograms, matrix effect data and storage stability data were not taken from a previously submitted ECM for soil: EPA MRID No. 49193107. Huang, S.-B. 2010. Fluazifop-P-Butyl: GRM044.03A - Analytical Method for the Determination of Fluazifop-P-Butyl (R154875; PP5), Fluazifop-P-Acid (R156172) and Compound X (R154719; CGA142110) in Soil Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS): Analytical Method Amendment. Report No.: GRM044.03A. Task No.: TK0019659. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 74 pages. Final report issued September 27, 2010. This soil method ECM used two loam soils, one sandy loam soil and a sand soil for validation.

11. It was reported for the ILV that one batch of thirteen samples required one working day with LC/MS/MS performed overnight (p. 30 of MRID 49800201).

V. References

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

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

Attachment 1: Chemical Names and Structures

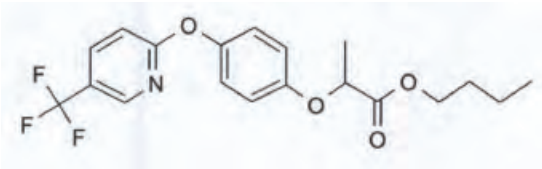
Fluazifop-P-Butyl (racemic mixture); R117009; CGA128175; PP9; R224237

IUPAC Name: (R,S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester

CAS Name: Not reported

CAS Number: 69806-50-4

SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)OCCCC)cc2



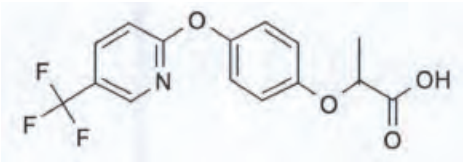
Fluazifop (racemic mixture); Fluazifop-P-Acid; R115625; CGA85619; PP6

IUPAC Name: (R,S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid

CAS Name: 2-[4-[[5-(Trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid

CAS Number: 69335-91-7

SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)O)cc2



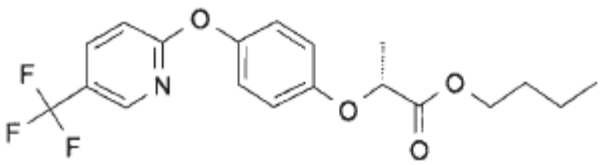
Fluazifop-P-Butyl (R enantiomer); R154875; CGA149108; PP5

IUPAC Name: (R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
Butyl (R)-2-{4-[5-(trifluoromethyl)-2-pyridyloxy]phenoxy}propionate

CAS Name: Butyl (2R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate

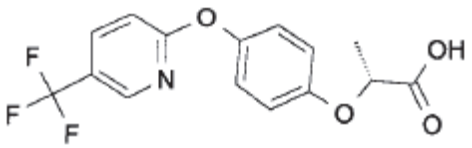
CAS Number: 79241-46-6

SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)OCCCC)cc2



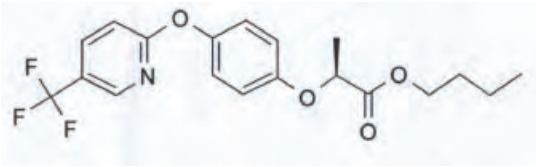
Fluazifop (R enantiomer); Fluazifop-P-Acid; R156172

IUPAC Name: (R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
CAS Name: Not reported
CAS Number: 83066-88-0
SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)O)cc2



Fluazifop-P-Butyl (S enantiomer); R159618

IUPAC Name: (S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
CAS Name: Not reported
CAS Number: Not reported
SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)OCCCC)cc2



Fluazifop (S enantiomer); Fluazifop-P-Acid; R159697

IUPAC Name: (S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
CAS Name: Not reported
CAS Number: 95977-30-3
SMILES String: n1cc(C(F)(F)F)ccc1Oc2ccc(OC(C)C(=O)O)cc2