Analytical method for fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) in soil

Reports: ECM: MRID 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.

ILV: MRID 49193106. Perez, R., J. L. Schmitt. 2013. Fluazifop-P-Butyl: Fluazifop-P-Butyl - Independent Laboratory Validation of Analytical Method (GRM044.03A) for the Determination of Fluazifop-P-Butyl (R154875; PP5), Fluazifop-P-Acid (R156172), Compound X (R154719; CGA142110) in Soil Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS): Final Report. Report and Task No.: TK0114928. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 160 pages. Final report issued February 6, 2013.

Document No.: MRIDs 49193107 & 49193106

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA or OECD Good Laboratory Practice (GLP) standards (p. 3). 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 authorization of revisions to previous method version was included (p. 5).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (p. 3 of MRID 49193106). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 of MRID 49193106). An authenticity statement was included with the quality assurance statement.

Classification: This analytical method is classified as ***supplemental***. Recoveries were corrected in the ECM and ILV for negligible residues in the controls. The LOD was misreported. Representative chromatograms were not provided for three of the four soil matrices in the ECM report.

PC Code: 122809

Reviewer:

Edmund M. Wong
Environmental Chemist
U.S. EPA

Signature: Edmund Wong

Date: 08/27/2014

All page citations refer to the Amendment page numbers at the bottom of MRID 49193107 (ECM) unless noted otherwise

Executive Summary

This analytical method, Syngenta Crop Method GRM044.03A, is designed for the quantitative determination of fluazifop-P-butyl (R154875; PP5), fluazifop-P-acid (R156172) and Compound X (R154719; CGA142110) in soil using LC/MS/MS. The method is quantitative for all three analytes at the stated LOQ of 1.0 µg/kg (1.0 ppb). The ECM validated the method using sandy loam, sand and two loam soils; the ILV validated the method using clay loam soil with the first trial. The method specifies the correction of procedural recoveries for residues in the controls. The LOQ is less than the lowest toxicological level of concern in soil. No major modifications were made by the independent laboratory.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluazifop-P-Butyl, Fluazifop-P-Acid, and Compound X (CGA142110)	49193107	49193106		Soil	09/27/2010	Syngenta Crop Protection, LLC	LC/MS/MS	1.0 µg/kg

I. Principle of the Method

Soil samples were warmed to ambient temperature prior to experiment (p. 14). Samples of soil (20 ± 0.1 g) were weighed into 50-mL disposable plastic centrifuge tubes. After 20 minutes of equilibration of the fortification solution, the soil was extracted twice with acetonitrile:”10 mM” ammonium acetate buffer at pH 5.5 (50:50, v:v; 30 mL first extraction, 20 mL second extraction) by shaking at room temperature for 20 minutes (pp. 14, 17-18; Appendices 1-2, pp. 72-74). After centrifugation of the combined extracts, the volume was adjusted to 50 mL with ”10 mM” ammonium acetate buffer at pH 5.5. An aliquot (1 mL) was filtered with a 3-mL disposable syringe containing a PTFE syringe membrane filter (13 mm; 0.2 µm). A 250- µL aliquot of the filtered extract was diluted with 750 µL of (4x dilution) prior to analysis via LC/MS/MS.

Samples were analyzed for fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) by HPLC (Ascentis Express C8, 50 x 3.0 mm, 2.7 µm column) with a column filter (ColumnSaver) using a gradient mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) 0.1% formic acid in HPLC grade methanol [time ratio A:B; 0.0-0.5 min. 90:10, 1.5-4.0 min. 40:60, 4.5-6.5 min. 10:90, 6.6-7.5 min. 90:10] with mass spectrometry in positive ion or negative ion mode (Multiple Reaction Monitoring mode, MRM; pp. 20-22). Analytes were identified with two transitions, quantification and confirmation ion transitions. Positive mode was employed for fluazifop-P-butyl with transitions of 384.15→328.00 and 384.14→282.00 and Compound X (CGA142110) with transitions of 164.05→146.00 and 164.06→75.00. Negative mode was employed for fluazifop-P-acid with transitions of 326.06→254.00 and 326.07→226.00. Injection volumes were 50 µL. In the ILV, only the quantitative transition was monitored, and injection volume was 10 µL (pp. 13-14 of MIRD 49193106).

The LOQ for fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) was validated as 1.0 µg/kg in the ECM and the ILV (p. 26, Figure 2, p. 45 and Figure 12, p. 55; p. 9 and Appendix 6, pp. 154-159 of MRID 49193106). The LOD for all analytes was 0.5 µg/kg.

II. Recovery Findings

ECM (MRID 49193107): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) at the LOQ, 10×LOQ and 50×LOQ in loam soils from Nebraska and Illinois, sandy loam soil from Georgia and sand soil from California (Tables 1A-1B, p. 30; Tables 3-6, pp. 33-36). Confirmation ion results were comparable (Tables 8-11, pp. 38-41). All of the procedural recovery values were corrected for the average of the residues found in the controls (based on protocol and data in chromatograms of Georgia soil; no other chromatograms or raw data were provided; p. 23; Figures 5-21, pp. 48-64). The soils were fully characterized (Tables 1A-1B, p. 30).

ILV (MRID 49263806): Mean recoveries and RSDs were within guideline requirements for analysis of fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) at the LOQ and 10×LOQ in clay loam soil from Underwood Farm, Ohio (pp. 9, 11-12; Tables 3-5, pp. 20-22; Appendix 4, p. 151 of MRID 49193106). Recovery values were corrected for the average of the residues found in the controls; the only recovery values which were not corrected were those of Compound X due to absence of residues in the controls. Only the quantitative ion was monitored. The method was validated with the first trial (p. 9 of MRID 49193106).

Table 2. Initial Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
Loam Soil (RIMV00110-0001) from Nebraska (0-6")						
Quantitative ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	90.8-111	96.2	8.5	8.8
	10	5	88.1-96.4	92.1	3.4	3.7
	50	5	89.6-94.4	92.5	2.0	2.2
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	85.6-90.2	88.7	1.8	2.1
	10	5	89.9-92.7	91.3	1.3	1.4
	50	5	90.4-94.2	91.9	1.5	1.6
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	85.8-97.0	92.1	5.4	5.9
	10	5	84.2-89.5	86.9	2.5	2.8
	50	5	87.4-91.3	88.7	1.6	1.8
Confirmation ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	86.6-108	93.9	8.2	8.8
	10	5	88.3-95.6	92.2	2.8	3.0
	50	5	91.4-97.9	93.1	2.6	2.8
Fluazifop-P-Acid	1.0 (LOQ)	5	73.8-90.0	81.2	7.1	8.7

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
(R156172)	10	5	86.1-93.6	89.8	2.8	3.1
	50	5	88.8-96.5	91.3	3.1	3.4
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	83.0-94.8	89.2	4.8	5.4
	10	5	83.4-90.1	87.0	2.7	3.1
	50	5	86.9-91.6	89.3	1.8	2.0
Loam Soil (RIMV00110-0002) from Illinois (0-6”)						
Quantitative ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	93.6-99.6	97.4	2.7	2.8
	10	5	91.6-98.7	95.2	2.8	2.9
	50	5	92.2-98.2	95.3	2.7	2.9
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	89.2-98.0	93.8	3.4	3.7
	10	5	93.2-97.2	94.8	1.5	1.6
	50	5	91.4-96.9	93.0	2.2	2.4
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	81.5-92.9	88.5	4.3	4.8
	10	5	85.1-91.8	88.2	2.5	2.9
	50	5	85.2-90.1	88.2	2.1	2.4
Confirmation ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	92.3-101	96.6	3.2	3.3
	10	5	93.5-99.3	96.0	2.2	2.3
	50	5	94.5-103	97.9	3.9	3.9
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	75.2-87.4	82.4	5.0	6.1
	10	5	92.5-100	95.9	3.8	3.9
	50	5	91.5-96.6	94.1	1.9	2.0
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	85.4-95.2	89.1	3.9	4.4
	10	5	85.7-90.8	88.7	2.3	2.6
	50	5	86.6-91.2	88.3	1.9	2.1
Sandy Loam Soil (RIMV00110-0003) from Georgia (0-6”)						
Quantitative ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	103-111	106	3.7	3.5
	10	5	103-111	107	2.9	2.8
	50	5	103-110	106	2.7	2.5
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	93.8-101	96.4	2.7	2.8
	10	5	93.3-97.3	95.2	1.8	1.9
	50	5	93.4-97.6	95.2	1.6	1.6
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	83.8-103	92.2	7.9	8.6
	10	5	88.3-92.4	90.0	2.2	2.5
	50	5	90.7-93.5	92.1	1.1	1.1
Confirmation ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	96.5-113	105	6.0	5.7
	10	5	103-110	105	2.9	2.7
	50	5	100-108	104	3.0	2.9
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	82.2-105	91.2	10.0	11.0
	10	5	89.9-95.1	94.1	3.2	3.4
	50	5	93.4-97.3	95.1	1.4	1.5
Compound X	1.0 (LOQ)	5	94.0-103	98.1	3.5	3.5

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
(R154719; CGA142110)	10	5	89.0-95.5	92.0	2.4	2.6
	50	5	91.1-96.8	94.3	2.5	2.6
Sand Soil (RIMV00110-0004) from California (0-6")						
Quantitative ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	99.1-112	103	5.2	5.1
	10	5	99.0-106	104	2.9	2.8
	50	5	100-107	105	2.8	2.6
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	85.4-90.8	88.1	2.0	2.2
	10	5	90.8-95.0	93.1	1.7	1.8
	50	5	97.1-101	99.4	1.7	1.7
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	83.1-94.9	89.2	4.2	4.7
	10	5	79.0-89.7	84.4	4.7	5.6
	50	5	89.0-91.9	90.9	1.1	1.2
Confirmation ion						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	99.1-107	102	3.3	3.3
	10	5	98.7-107	103	3.1	3.0
	50	5	101-108	105	2.6	2.5
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	82.0-91.2	86.8	4.8	5.5
	10	5	91.2-95.8	93.9	2.1	2.3
	50	5	98.9-102	101	1.5	1.5
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	84.6-92.6	87.8	3.0	3.5
	10	5	82.3-93.6	86.9	4.6	5.3
	50	5	91.1-95.0	92.7	1.4	1.5

Data were obtained from Tables 3-6, pp. 33-36 and Tables 8-11, pp. 38-41 in the study report. All recovery values were corrected for the average of the residues found in the controls (based on protocol and data in chromatograms of Georgia soil; no other chromatograms or raw data were provided; p. 23; Figures 5-21, pp. 48-64).

Table 3. Independent Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil from Underwood Farm, Ohio						
Fluazifop-P-Butyl (R154875; PP5)	1.0 (LOQ)	5	110-117	114	2.8	2.4
	10	5	101-116	109	5.6	5.1
Fluazifop-P-Acid (R156172)	1.0 (LOQ)	5	67-83	75	5.7	7.5
	10	5	84-88	85	1.7	2.0
Compound X (R154719; CGA142110)	1.0 (LOQ)	5	81-89	83	3.5	4.2
	10	5	82-89	85	2.8	3.3

Data were obtained from pp. 9, 11-12; Tables 3-5, pp. 20-22; Appendix 4, p. 151 of MRID 49193106. Recovery values were corrected for the average of the residues found in the controls; the only recovery values which were not corrected were those of Compound X due to absence of residues in the controls.

III. Method Characteristics

The LOQ for fluazifop-P-butyl, fluazifop-P-acid and Compound X (CGA142110) was reported as 1.0 µg/kg in the ECM and the ILV (p. 26, Figure 2, p. 45 and Figure 12, p. 55; p. 9 and Appendix 6, pp. 154-159 of MRID 49193106). In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. The ECM study author also noted that the LOQ was a value which was no lower than four times the mean amplitude of the background noise of the untreated sample at the retention time of the analytes. The LOD for all analytes was 0.5 µg/kg in the ECM and the ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise of an untreated sample, as well as three times the background noise. The ECM study author noted that the LOD was approximately equivalent to half of the theoretical amount for a recovery sample at the method LOQ; however, the value was reported in terms of HPLC aliquot concentration rather than soil sample concentration. The ECM study author reported the LOD as 2.5 pg or 0.050 pg/µL. The ILV study author also reported the LOD as 0.050 ppb, the units of which are confusing. These aliquot concentrations represent a soil sample concentration of 0.5 µg/kg. The ECM study author also noted that LOD may vary based on the specific laboratory analytical instrument.

Table 4. Method Characteristics

	Fluazifop-P-Butyl (R154875; PP5)	**Fluazifop-P-Acid (R156172)**	**Compound X (R154719; CGA142110)**
Limit of Quantitation (LOQ)	1.0 µg/kg	1.0 µg/kg	1.0 µg/kg
Limit of Detection (LOD)	0.50 µg/kg	0.50 µg/kg	0.50 µg/kg
Linearity (calibration curve r^2 and concentration range)	r^2 = 0.9993-0.9998[1] (0.05-10 pg/µL)	r^2 = 0.9999[1] (0.05-10 pg/µL)	r^2 = 0.9999[1] (0.05-10 pg/µL)
Repeatable	Yes	Yes	Yes
Reproducible	Yes[2]	Yes[2]	Yes[2]
Specific	Yes	Yes	Yes

Data were obtained from pp. 19, 26-27; Figure 2, p. 45; Figure 12, p. 55; Figures 22-27, pp. 65-70 of the study report.

1 Calibration curves were reported for the quantification and confirmation ion transitions; no soil matrix was specified. ILV calibration curves were linear, r^2 = *ca*. 0.9986-0.9994 for all three analytes, for concentration range of 0.5-100 pg (see Figures 37-39, pp. 60-62 and Appendix 6, pp. 157-159 of MRID 49193106). ILV calibration curves were calculated for the quantitative ion transition only. Reviewer-calculated calibration curves verified linearity for the ILV (r^2 = 0.9995-0.9998 for all three analytes in clay loam soil; reviewer-calculated values contain a degree of uncertainty due to poor resolution of the study report; see DER Attachment 2). Individual calibration data was not reported in the ECM.

2 The ECM validated the method using four soils (sandy loam, loam and sand soils); the ILV validated the method using one soil (clay loam soil).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, residues in the control samples were less than 20% of the LOD (based on representative chromatograms of the Georgia soil; p. 27; Figure 4, p. 47; Figure 11, p. 54; Figure 18, p. 61). In the ILV, the residues which were found in the control samples ranged from 0% to *ca*. 10% at the LOQ (Appendix 6, pp. 157-159 of MRID 49193106). The ECM study author reported that these interfering residues were due to residual analyte carryover and minor chromatographic and isobaric interferences (pp. 24-25, 27).

The independent laboratory used matrices supplied by the registrant rather than independently sourced matrices. Guideline 850.6100 says that the independent laboratory “verifies that matrix control samples are free of interferences at the appropriate retention time, wavelength or detector setting” and that “interferences with peak areas that are less than 50 percent (%) at the MDL or LOD, are considered not significant.” The independent laboratory could have used independently sourced matrices and further limited interferences.

2. Recovery values were corrected for residues found in the controls in both the ECM and ILV reports. Guideline 850.6100 says that “data from matrix control samples (blanks) are not used to correct values from spiked matrix controls for recoveries.” In the ECM, all procedural recovery values were corrected for the average of the residues found in the controls (based on protocol and data in chromatograms of Georgia soil; no other chromatograms or raw data were provided; p. 23; Tables 3-6, pp. 33-36; Tables 8-11, pp. 38-41; Figures 5-21, pp. 48-64). In the ILV, the only recovery values which were not corrected were those of Compound X due to absence of residues in the controls (p. 9; Tables 3-5, pp. 20-22; Figures 31-36, pp. 54-59 of MRID 49193106). It is unclear why both laboratories corrected recoveries for negligible carryover residues.

3. The LOD was reported in terms of HPLC aliquot concentration rather than soil sample concentration. The ECM study author reported the LOD as 2.5 pg or 0.050 pg/µL. The ILV study author also reported the LOD as 0.050 ppb, the units of which are confusing. These aliquot concentrations represent a soil sample concentration of 0.5 µg/kg.

4. In the ECM, sample chromatograms are only provided for Georgia control soil, reagent blanks, and calibration standard solutions (Figures 1-21, pp. 44-64). No representative chromatograms were provided for the control soils from Illinois, Nebraska, and California. No representative chromatograms were provided for any spiked samples.

5. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in one working day, not including LC/MS/MS (p. 15 of MRID 49193106).

6. The ILV concluded that the method was adequate as written (p. 15 of MRID 49193106).

V. References

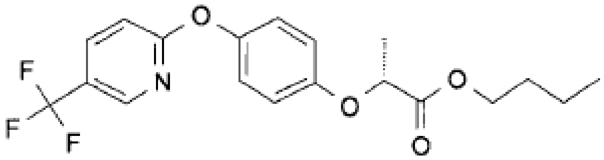
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

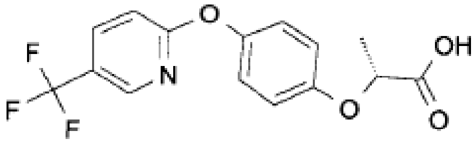
Fluazifop-P-Butyl; R154875; PP5

IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxyl]-propionic acid butyl ester.
CAS Name:	(2R)-2-[4-[[5-(Trifluoromethyl)-2-pyridinyl]oxy]phenoxyl]-propionic acid butyl ester.
CAS Number:	79241-46-6
SMILES String:	Not reported



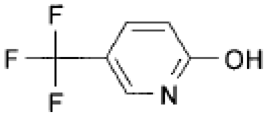
Fluazifop-P-Acid; R156172

IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxyl]-propionic acid.
CAS Name:	(2R)-2-[4-[[5-(Trifluoromethyl)-2-pyridinyl]oxy]phenoxyl]-propionic acid.
CAS Number:	83066-88-0
SMILES String:	Not reported



Compound X; R154719; CGA142110

IUPAC Name:	5-Trifluoromethyl-pyridin-2-ol.
CAS Name:	5-(Trifluoromethyl)-2(1H)-pyridinone.
CAS Number:	33252-63-0
SMILES String:	Not reported



Test Material: Fluazifop-P-Butyl

MRID: 49193107

Title: 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

MRID: 49193106

Title: Fluazifop-P-Butyl: Fluazifop-P-Butyl - Independent Laboratory Validation of Analytical Method (GRM044.03A) for the Determination of Fluazifop-P-Butyl (R154875; PP5), Fluazifop-P-Acid (R156172), Compound X (R154719; CGA142110) in Soil Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS): Final Report

EPA PC Code: 122809

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 7/8/14

Secondary Reviewer: Dan Hunt **Signature:** Dan Hunt

Date: 7/8/14

QC/QA Manager: Joan Gaidos **Signature:**

Date: 7/8/14