2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta analytical method GRM 044.05A, entitled "Fluazifop-P-Butyl - Analytical Method for Enantiomeric Ratio and Residue Determination of Fluazifop-P-Butyl (R154875; PP5) and its Acid Degradate (R156172) in Soil".

This study was designed to satisfy harmonized guideline requirements described in EPA OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods), EC Guidance Documents SANCO/3029/99 Rev.4 (2000), and SANCO/825/00 Rev.8.1 (2010). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

BRIEF SUMMARY OF METHOD

R224237and CGA85619 are analyzed by direct injection of soil samples using ultra performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ of the method is 0.001 mg/kg for individual enantiomers.

For analysis of R224237and CGA85619, 200 µL of 0.2 µg/mL(LOQ) or 400 µL of 1 µg/mL (10XLOQ) was spiked into the 20 g soil sample. 30 mL extraction solution was added to the tube, the supernatant was transferred to a clean 50 mL tube after shaking and centrifuge. 20 mL ectraction solution was added to the tube to repeat the extraction. The supernatant was combined and the final volume was adjusted to 50 mL with Buffer A after shaking and centrifuge. 1 mL of the resulting extract was filtered. An aliquot of 500 µL of the sample was added into HPLC vial with 500 µL of Buffer A. The sample was injected for analysis using LC-MS/MS.

Twenty (20) g sub-samples of soil were extracted two times by mechanical shaking (20 minutes) with acetonitrile: Buffer A adjusted to pH 5 (50:50; v/v). The extracts were combined upon centrifugation and the extraction volume adjusted to 50 mL. Aliquots were syringe filtered (0.2µm PTFE). Final determination is carried out by ultra performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) using electrospray ionization techniques.

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

The test/reference substances were obtained from Syngenta Crop Protection, LLC. The following test/reference substances were used:

ANALYTES	STANDARD	LOT NUMBER	PURITY (%)	EXPIRATION DATE	STORAGE
CGA85619	RS15194	DAH-XXXI-1	99.0	May 31, 2016	Refrigerator
R224237	RS15193	ST-I-4	99.0	August 31, 2017	Refrigerator
R154875	RS15196	ASJ10061-05	94.2	August 31,2016	Refrigerator
R156172	RS15195	ARS-I-15	99.5	July 31, 2017	Refrigerator

Note: The S-isomers were not provided by Syngenta. The R-isomer was used to establish retention time and the racemic mixture was used to establish the S-isomer retention time for proper identification (See Appendix 5, Communications).

The detailed information is listed in Figures 1-4.
Characterization data for the test/reference standards are maintained by Syngenta Crop Protection, LLC. The Certificate of Analysis is included in Appendix 3.

3.2 Test System

The test system evaluated for this ILV was Clay Loam Soil (CAPY081512) and Sandy Loam Soil (EXCEL FARM). This matrix was chosen because it is representative of the matrices the method was designed for. The control samples used in this study were provided by Syngenta Crop Protection, LLC. These control soil samples were characterized by AGVISE Laboratories of Northwood, North Dakota. GLP characterization results in more detail are presented in Table 1 and summarized below:

USDA Textural Class	Sample ID	pH	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)	Hydrogen
Clay Loam	CAPY081512	6.9	1860	1080	47	272	29
Sandy Loam	EXCEL FARM	7.8	1191	233	48	142	11

3.3 Equipment and Reagents

The equipment and reagents used for this study were selected and prepared as outlined in the method. Identical or equivalent equipment and materials may have been used, as permitted by the method.

3.3.1 Equipment

Equipment	Description	Supplier
General lab glassware	General lab glassware	Thermoscientific
General lab plastic ware	General lab plastic ware	Thermoscientific
Autosampler vials	Snap cap, 2 mL size	Thermoscientific
LC-MS/MS system Including HPLC and autosampler units	Acquity UPLC system; AB Sciex 6500 triple quadrupole mass spectrometer with Analyst TM software version 1.6.2	Waters Corporation Applied Biosystems
HPLC column	Chiralpak AS-RH, 4.6 x 150 mm, 5.0µm	Chiral
Centrifuge Devices	Allegra 6KR Centrifuge	Beckman Coulter
Balance	Mettler Toledo, M72237	Mettler Toledo
Balance	Sartorius, LC220S	Sartorius

3.3.2 Reagents

Reagent	Description	Supplier
Water	In house	Milli-Q Water
Ammomium hydroxide	ACS grade	Sigma Aldrich
Formic acid	ACS grade	Sigma Aldrich
2-Propanol	ACS grade	BDH
Acetonitrile	HPLC grade	PHARMCO-AAPER
Ammonium acetate	ACS grade	Alfa Aesar
Acetic acid	ACS grade	Fisher
Methanol	HPLC grade	J.T. Baker
CGA85619, R224237, R156172 and R154875	GLP certified	Syngenta Crop Protection, LLC P.O Box 18300 Greensboro, NC 27419-8300

3.3.3 Preparation of Reagents

a) 10% Ammonium Hydroxide; prepared by mixing 10mL Ammonium Hydroxide with 90mL Milli-Q Water.

b) Methanol/Water, 50/50 (v/v); prepared by mixing 500 mL HPLC grade methanol with 500 mL Milli-Q water.

c) "Buffer A"; 10mM ammonium acetate buffer at *p*H 5.0, prepared by mixing 2 L of Milli-Q water, 1.5418 g of ammonium acetate, 1.14 mL of acetic acid. Using a calibrated pH meter, adjusted the pH to 5.0 using dropwide additions of a 10% ammonium hydroxide (NH_4OH) solution.

d) Acetonitrile/Buffer A, 50/50 (v/v); prepared by mixing 500 mL HPLC grade acetonitrile with 500 mL Buffer A.

e) Acetonitrile/Buffer A, 30/70 (v/v); prepared by mixing 300 mL HPLC grade acetonitrile with 700 mL Buffer A.

f) "Mobile Phase A"; 0.1% Formic acid in water with 5% 2-propanol, prepared by mixing 950 mL of Milli-Q water, 1.0 mL of Formic acid and 50 mL of 2-propanol.

g) "Mobile Phase B"; 0.1% Formic acid in acetonitrile with 5% 2-propanol, prepared by mixing 950 mL of Milli-Q water, 1.0 mL of Formic acid and 50 mL of 2-propanol.

3.4 Preparation of Standard Solutions

3.4.1 Stock Standard Solutions

Prepare individual 100 µg/mL stock solutions for fluazifop-butyl (R224237; racemic mixture), fluazifop (CGA85619; racemic mixture), fluazifop-P-butyl (R154875; *R*-isomer enriched), fluazifop-P (R156172; *R*-isomer enriched). Weigh out accurately, using a five figure balance, sufficient individual analytical standards into a "Class A" volumetric flask (100 mL; amber). Dilute to the mark with acetonitrile.

Example of Stock Standard Solution Preparation

Compound	Weight (g)	Diluted To (mL)	Concentration (µg/mL)	Standard Solution ID
R224237	0.005587	50	110.62	S082715-1
CGA85619	0.005560	50	110.08	S082715
R154875	0.011749	100	110.68	S082715-3
R156172	0.015056	100	149.8	S082715-2

3.4.2 Fortification Standards

3.4.2.1 Fortification solutions containing R224237 at 10.0 µg/mL, 1.0 µg/mL and 0.2 µg/mL

Transfer 2.260 mL of the R224237 standard stock solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 10.0 µg/mL R224237 solution.

Transfer 2.5 mL of the 10.0 µg/mL R224237 solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 1.0 µg/mL R224237 solution.

Transfer 5.0 mL of the 1.0 µg/mL R224237 solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 0.2 µg/mL R224237 solution.

3.4.2.2 Fortification solutions containing CGA85619 at 10.0 µg/mL, 1.0 µg/mL and 0.2 µg/mL

Transfer 2.271 mL of the CGA85619 standard stock solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 10.0 µg/mL CGA85619 solution.

Transfer 2.5 mL of the 10.0 µg/mL CGA85619 solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 1.0 µg/mL CGA85619 solution.

Transfer 5.0 mL of the 1.0 µg/mL CGA85619 solution into a 25 mL volumetric flask and dilute to the mark with acetonitrile to yield a 0.2 µg/mL CGA85619 solution.

3.4.3 Calibration Standards

The working calibration standards are prepared individually from the 110 µg/mL stock standards by transferring 2.3 mL of the appropriate stock solutions into a 25-mL volumetric flask and diluting to the mark with acetonitrile resulting in intermediate working standards at 10 µg/mL concentration levels in acetonitrile. Similarly, further dilution of these working standards, individually, with acetonitrile: Buffer A (30:70; v/v) results in intermediate working standards at 1.0 µg/mL concentration levels. The two sets of LC-MS/MS calibration standards (R224237and CGA85619) are prepared by serial dilutions of the intermediate working standards at 1.0 µg/mL concentration levels in acetonitrile:10mM ammonium acetate pH 5 (30:70; v/v). Eight concentration levels, at a range from 0.10 to 20 pg/µL (equivalent to 0.05 to 10 pg/µL of individual enantiomers) should be prepared.

Example of Intermediate Calibration Solution Preparation

Stock Solution			Intermediate Calibration Solution				
Standard Solution ID	Compound	Conc. (µg/mL)	Aliquot Taken (µL)	Diluted To (mL)	Solvent	Conc. (ng/mL)	Intermediate Calibration Solution ID
S082715-1	R224237	110.62	2.260	25	ACN	10	F082715-4
S082715	CGA85619	110.08	2.271	25	ACN	10	F082715-1

Example of Calibration Standard Solution Preparation

Intermediate Calibration Solution			Calibration Solution				
Intermediate Calibration Solution ID	Compound	Conc. (ng/mL)	Aliquot Taken (mL)	Diluted To (mL)	Solvent	Conc. (ng/mL)	Calibration Solution ID
F082715-3	CGA85619	200	5	50	ACN:Buffer A (30:70; v/v)	20.0	C082715-1
F082715-3	CGA85619	200	2.5	50		10.0	C082715-2
C082715-2	CGA85619	10	10	20		5.0	C082715-3
C082715-1	CGA85619	20	2.5	25		2.0	C082715-4
C082715-2	CGA85619	10	2.5	25		1.0	C082715-5
C082715-3	CGA85619	5	5.0	50		0.5	C082715-6
C082715-4	CGA85619	2	5.0	50		0.2	C082715-7
C082715-5	CGA85619	1	5.0	50		0.1	C082715-8

3.5 Analytical Procedures and Modifications

3.5.1 Modifications

Modifications were made to the method with regard to the Buffer A preparation and mobile phase gradient.

3.5.1.1 Mobile Phase Composition

Mobile Phase B, which is in constant composition during the run, was added to Mobile Phase A and C at 5% each. In this way, the gradient shown below was adjusted accordingly to remain the same composition as stated in the method.

Time (min)	%A	%C	Flow Rate (µL/min)
0.0	52.6	47.4	600
3.0	52.6	47.4	600
4.0	36.8	63.2	600
12.0	36.8	63.2	600
12.1	52.6	47.4	600
13.0	52.6	47.4	600

3.5.1.2 Buffer A preparation

Buffer A was prepared by mixing 2L of HPLC water with 1.5422g ammonium acetate and 1.14 mL of glacial acetic acid. Then using a calibrated pH, ajusted the pH to 5.02 using dropwise additions of a 10% ammonium hydroxide (NH_4OH) solution.

3.5.2 Sample Preparation and Fortifications

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples were included with each sample set. To each pre-weighed control soil sample, appropriate amount of standard solutions containing analytes of interest (fluazifop-butyl and fluazifop) in acetonitrile was added. Each sample was standing for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. One untreated control and two fortified control samples were analysed with each sample set to verify method performance.

Details about the fortifications are summarized as follows:

Matrix	Compounds	Fortification Level	Fortification Vol. (mL)	Fortification Conc. (µg/mL)	Sample Vol. (mL)	Dilution Factor	Final Conc. (µg/L)	Rep.
Sandy Loam Or Clay Loam Soil	R224237 or CGA85619	LOQ	0.2	0.2	50	2	0.4	5
		10X LOQ	0.4	1.0	50	2	4	5

3.5.3 Sample Preparation

Twenty (20) g sub-samples of soil were extracted two times by mechanical shaking (20 minutes) with acetonitrile:Buffer A adjusted to pH 5 (50:50; v/v). 30 mL extraction solution was added to the tube, the supernatant was transferred to a clean 50 mL tube after shaking and centrifuge. 20 mL extraction solution was added to the tube to repeat the extraction. The supernatant was combined and the final volume was adjusted to 50 mL with Buffer A after shaking and centrifuge. 1 mL of the resulting extract was filtered. An aliquot of 500 µL of the

sample was added into HPLC vial with 500 µL of Buffer A. The sample was injected for analysis using LC-MS/MS.

3.6 Instrumentation

3.6.1 LC-MS/MS Instrument Description for Analysis of R224237 and CGA85619

LC System:	Waters Acquity UPLC system
MS Detector:	Applied Biosystems Sciex API 6500 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2

Chromatographic Conditions

Flow Rate:	0.6 mL/min
Column:	Chiralpak AS-RH, 4.6 x 150 mm, 5.0 mm
Injection Vol.:	50 µL
Run Time:	13 minutes
Mobile Phase A:	0.1% Formic acid in water with 5% 2-propanol
Mobile Phase B:	0.1% Formic acid in acetonitrile with 5% 2-propanol

Isocratic/Gradient Flow:

Time	% A	% B	Flow Rate (mL/min)
0	52.6	47.4	0.6
3.0	52.6	47.4	0.6
4.0	36.8	63.2	0.6
12.0	36.8	63.2	0.6
12.1	52.6	47.4	0.6
13.0	52.6	47.4	0.6

Approximate Retention Times of Analytes:

Analyte	Retention Time (min)
R224237	S:9.82 R:10.31
CGA85619	S:5.70 R:6.36

Mass Spectrometer Conditions

Interface:	TurboIon Spray	
Polarity:	Negative	Positive
Curtain gas (CUR):	30	30
Temperature (°C)	300	300
Ionspray voltage	4000	-3200

Collision gas setting (CAD)	8	8
Gas 1 (GS1)	45	45
Gas 2 (GS2)	50	50
Interface heater (ihe)	tuned value(s)	tuned value(s)
Scan type:	MRM	MRM

Analyte	MS/MS Transition	Dwell (ms)	DP	EP	CE	CXP
S- Enantiomer in R224237: ESI Positive						
Primary	384.101→282.100	150	70	10	22	12
Confirmatory	384.101→328.100	150	70	10	18	12
R- Enantiomer in R224237: ESI Positive						
Primary	384.101→282.100	150	70	10	22	12
Confirmatory	384.101→328.100	150	70	10	18	12
Analyte	MS/MS Transition	Dwell (ms)	DP	EP	CE	CXP
S- Enantiomer in CGA85619: ESI Negative						
Primary	326.100→254.100	150	-70	-10	-16	-12
Confirmatory	326.100→226.100	150	-70	-10	-31	-12
R- Enantiomer in CGA85619: ESI Negative						
Primary	326.101→254.100	150	-70	-10	-16	-12
Confirmatory	326.101→226.100	150	-70	-10	-31	-12

Data Acquisition

The peak integration and peak area count quantitation were performed by PE Sciex Analyst® (Version 1.6.2). A best-fit linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The correlation coefficient (r) for the calibration curves for each analytical set was greater than or equal to 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations, and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package.

FIGURE 1 R224237

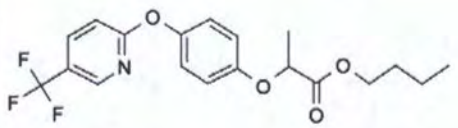
Compound Structure	
Syngenta Code Number:	PP9
Alternative compound code number:	R224237; CGA128175
CAS Number:	69806-50-4
Molecular Weight:	383.36
Molecual Mass	383.13
IUPAC Name:	(R,S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
Molecular Formula:	$C_{19}H_{20}F_3NO_4$
Standard Reference:	Syngenta Crop Protection LLC
Storage Conditions:	Refrigerator
Lot Number:	ST-I-4
Purity:	99.0%
Expiration Date:	08/31/2017

FIGURE 2 CGA85619

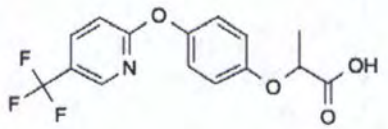
Compound Structure	
Syngenta Code Number:	PP6
Alternative compound code number:	R115625; CGA85619
CAS Number:	69335-91-7
Molecular Weight:	327.25
Molecual Mass	327.07
IUPAC Name:	(R,S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
Molecular Formula:	$C_{15}H_{12}F_3NO_4$
Standard Reference:	Syngenta Crop Protection LLC
Storage Conditions:	Refrigerator
Lot Number:	DAH-XXXI-1
Purity:	99.0%
Expiration Date:	05/31/2016

FIGURE 3 R154875

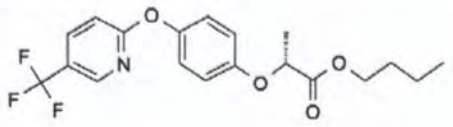
Compound Structure	
Syngenta Code Number:	PP5
Alternative compound code number:	R154875; CGA149108
CAS Number:	79241-46-6
Molecular Weight:	383.36
Molecual Mass	383.13
IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
Molecular Formula:	$C_{19}H_{20}F_3NO_4$
Standard Reference:	Syngenta Crop Protection LLC
Storage Conditions:	Refrigerator
Lot Number:	ASJ10061-05
Purity:	94.2%
Expiration Date:	08/31/2016

FIGURE 4 R156172

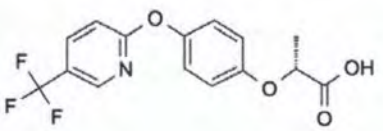
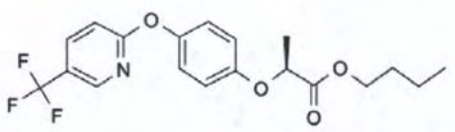
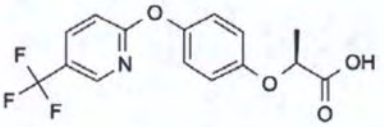
Compound Structure	
Syngenta Code Number:	R156172
Alternative compound code number:	N/A
CAS Number:	83066-88-0
Molecular Weight:	327.25
Molecual Mass:	327.07
IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
Molecular Formula	$C_{15}H_{12}F_3NO_4$
Standard Reference:	Syngenta Crop Protection LLC
Storage Conditions:	Refrigerator
Lot Number:	ARS-I-15
Purity:	99.5%
Expiration Date:	07/31/2017

FIGURE 5 R159618

Compound Structure	
Syngenta Code Number:	R159618
Alternative compound code number:	N/A
CAS Number:	N/A
Molecular Weight:	383.36
Molecual Mass	383.13
IUPAC Name:	(S)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
Molecular Formula:	C_{19} H_{20} F_3 N O_4
Standard Reference:	Syngenta Crop Protection LLC (Did not provide by Syngenta)

Note: The substrate was not provided.

FIGURE 6 R159697

Compound Structure	
Syngenta Code Number:	R159697
Alternative compound code number:	N/A
CAS Number:	95977-30-3
Molecular Weight:	327.25
Molecual Mass	327.07
IUPAC Name:	Syngenta Crop Protection LLC (Did not provide by Syngenta)

Note: The substrate was not provided.

STUDY OBJECTIVE

Syngenta Analytical Method GRM044.05A (Reference 1) was designed/developed for the enantiomeric ratio and residue determination of fluazifop-butyl (R and S) and its acid metabolite fluazifop (R and S) in soil. The limit of quantitation (LOQ) of the method has been proposed at 0.001 mg/kg (1 ppb) for individual enantiomers.

The objectives of this independent laboratory validation (ILV) study are:

(1) To provide validation data, i.e. recovery rates (accuracy) and relative standard deviation (precision) per the analytical procedures outlined in the method.

(2) To demonstrate the linearity of calibration curves.

The current guidance documents for this study are available in the Reference Section.

REFERENCE STANDARDS

Reference standards will be supplied by Syngenta Crop Protection, LLC. A Material Safety Data Sheet (MSDS) should accompany the reference standard, if available. The relevant information of the standards will be included in the report.

MATERIALS/EQUIPMENT

In this study, an LC-MS/MS system will be used for the determination of residues in sample final fractions with two MRM transitions monitored, i. e. both the primary and confirmatory transitions as specified in the method. The sources and grades of the solvents and chemical reagents will be selected per the requirements of the method. If replacements have to be made, the sources, grades and part numbers will be documented in the study records.

TEST SYSTEM

The test system for this study will consist of two types of soils. The untreated control sample (UTC) will be provided by the Study Sponsor, along with the characterization information.

SAMPLE	MATRIX	SAMPLE DESCRIPTION
PASC ID: 150304-1	Clay Loam	Percent Sand: 29% Percent Silt: 36% Percent Clay: 35%
PASC ID: 150304-2	Sandy Loam	Percent Sand: 73% Percent Silt: 16% Percent Clay: 11%

The soil sample were stored frozen after arrival at PASC, and will be taken out prior to analysis.

EXPERIMENTAL DESIGN

Prior to conducting sample analysis, PASC will establish method control not limited to but including analyte retention time, linearity, instrument response, instrument detection limits, procedures, and verification that the control soil matrix is free of interferences. PASC will demonstate method control by performing assessment tests before proceeding to method validation trials. More than one assessment test may be made depending on the number and type of substitutions. Data and results of any assessment test shall be included in the study records, but not in the final report.

Clarification or interpretation of the method will be provided by the sponsor or method developer if requested by the Study Director. All contacts made during the establishment of the method will be documented in writing by the performing laboratory and presented in the final report.

Control soil samples will be fortified with known amounts of individual analyte (PP9 or PP6) and analyzed per the procedures outlined in the method (Reference 1).

One validation set should include:

1x Reagent Blank (matrix free sample submitted to procedures outlined in method)

2x Control Samples (untreated control soil)

5x LOQ Recovery Samples (5 replicates at the target LOQ)

5x 10X LOQ Recovery Samples (5 replicates at 10X the target LOQ)

PASC should verify that the matrix control materials are free of interferences at the appropriate retention time or detector setting by examining the control samples under the instrumental conditions specified in the method. A response greater than 30% of each proposed LOQ constitutes a significant interference. If this is observed, the Study Monitor will be contacted for direction on how to proceed.

METHOD PERFORMANCE

A successful ILV will require performance data on at least one complete set of samples meeting the necessary criteria described in guidelines EPA OCSSP 850.6100 (2012), EC SANCO/3029/99 Rev.4 (2000), and SANCO/825/00 Rev.8.1 (2010) of that matrix type. Generally, this requires mean recoveries to be within 70 -110% for that matrix type. The relative standard deviation of replicate measurements of recoveries should be $\leq$ 20% at or above the method LOQ. Upon successful completion, the Study Director or delegate will proceed to write the final report. If the mean results fall outside the 70% to 110% range, the Study Director must consult the Study Sponsor to determine if further trials are required. A maximum of t hree (3) validation trials for that matrix type may be analyzed to show the subject method is valid.

Communication between the Study Director and the Study Sponsor should occur after each validation set is analyzed, and must be fully documented in the study records.

PROPOSED STATISTICAL METHODS

The statistical method for a regression analysis of a standard curve and quantification of residues are described in the method. The accuracy will be determined in terms of a mean and standard deviation for the recovery results from this study. Precision will be demonstrated by calculating the mean, range, standard deviation, and relative standard deviation of the sample recoveries.

MODIFICATIONS

During the 1st method trial, the method procedure should be followed as written. However, if the performance data on the first attempt is unsuccessful, the independent laboratory may contact the Study Sponsor to clarify the directions given in the method. Any correspondence must be documented in writing in the final report. If the second attempt is unsuccessful, the performing laboratory may contact the study sponsor for further clarification. Failure of the third attempt will terminate the study and a report will be issued to the study sponsor, Syngenta, explaining why the method failed.

ROUTE OF ADMINISTRATION

The test and reference material will be prepared as outlined in the analytical method.

JUSTIFICATION OF THE TEST SYSTEM

The control samples will be analyzed with the method for evaluation of substrate-related interferences, and the fortified samples will be analyzed using the method for evaluation of method performance via procedural recoveries.

CONTROL OF BIAS

Bias will be controlled by experimental design and statistical methods performed on procedural recoveries from a homogeneous mixture by fortification.

RECORDS TO BE MAINTAINED

All personnel involved in this study will maintain laboratory notebooks and or worksheets for recording data as required by Good Laboratory Practice regulations. All original raw data, final report, correspondence, protocol, protocol amendments, and all pertinent information generated during this study will be transferred to Syngenta Crop Protection, PO Box 18300, Greensboro, NC 27419 for final archive.

PROTOCOL

Upon Study Sponsor approval, all changes in or revisions with reasoning, of an approved protocol will be documented, dated, and signed by the Study Director and Study Sponsor. All changes or revisions will be maintained with the original protocol.

SPECIMEN DISPOSAL

Samples will be retained at the performing laboratory until discard approval is received from the Study Sponsor or Testing Facility Management.

CONFIDENTIALITY STATEMENT

The analytical methods and the results of this study are the sole property of Syngenta and shall be kept confidential by the performing laboratory.

GLP COMPLIANCE

All aspects of this study shall be performed and reported in compliance with U.S. EPA Good Laboratory Practice (GLP) Standards (40 CFR Part 160), which are compatible with the OECD Principles of Good Laboratory Practice (as revised in 2007). The Test Facility's final report or data package (supplied to the Sponsor) shall contain a statement that the study was conducted in compliance with current and applicable GLP standards and will outline, if appropriate, any deviations in the study from those standards. This statement shall be signed by the Study Director (or responsible party at the Test Facility).

FINAL REPORT

Upon successful or unsuccessful validation of the method, the performing laboratory will submit results of the ILV to the Study Sponsor in the form of a final report. Refer to the guidance documents cited in preparation of the final report.

REFERENCES

1. Huang Sung-Ben, "Amended Analytical Method for Enantiomeric Ratio and Residue Determination of Fluazifop-P-Butyl (R154875; PP5) and its Acid Degradate (R156172) in Soil"

2. United States Environmental Protection Agency, Office of Prevention, Pesticide and Toxic Substances, Ecological Effects Test Guidelines EPA Documents EPA 712-C-001 (OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation, Jan, 2012. Internet address: http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm

3. European Commission, Directorate General Health and Consumer Protection, (SANCO/825/00 rev.8.1) Guidance Document on Pesticide Residue Analytical Methods, 16/11/2010. Internet Address: http://ec.europa.eu/food/plant/plant_protection_products/guidance_documents/docs/guide_doc_825-00_rev8_en.pdf

4. European Commission, Directorate General Health and Consumer Protection, (SANCO/3029/99 rev.4) Residues: guidance for Generating and reporting methods of analysis in Support of Pre-Registration Data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, 11/07/00. Internet Address: http://ec.europa.eu/food/plant/plant_protection_products/guidance_documents/docs/wrkdoc12_en.pdf