2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta Analytical Method GRM044.03A entitled "Fluazifop-P-Butyl – Independent Laboratory Validation of Analytical Method (GRM044.03A) 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)" as performed by ADPEN Laboratories, Inc.

This study was designed to satisfy harmonized guideline requirements described in OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods) and Organization for Economic Co-Operation and Development (OECD), Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17. This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

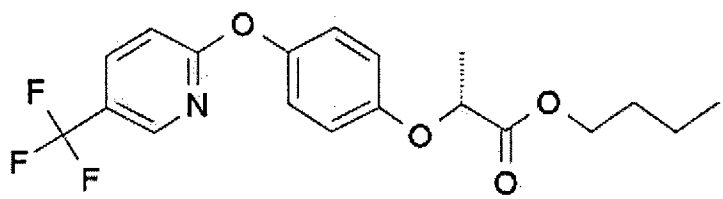
3.0 MATERIALS AND METHODS

3.1 Reference Substances

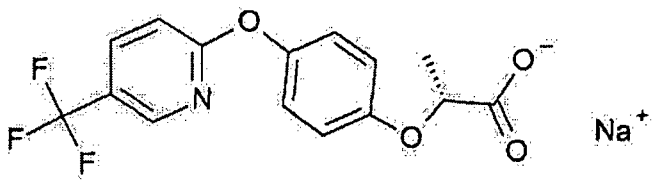
The reference substances were obtained from Syngenta Crop Protection and stored as directed. All fortification and calibration solutions made from the reference substances (analytical standards) were stored according to the method.

The following reference substances were used:

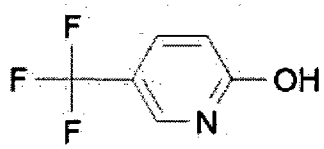
Common Name: **Fluazifop-P-Butyl**
IUPAC Name: (R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
CAS Number: 79241-46-6
Molecular Formula: $C_{19}H_{20}F_3NO_4$
Molecular Weight: 383.3 g/mol
Storage Conditions: Refrigerator
Batch Identification: ASJ10030-06
Purity: 93.4%
Reanalysis Date: July 2013
Structure:



Common Name: **Fluazifop-P Acid**
Synonyms: CSCC890014, R156172 (received as sodium salt)
CAS Number: 83066-88-0 (Fluazifop-P acid)
Molecular Formula: $C_{15}H_{11}F_3NO_4Na$
Molecular Weight: 349.2 g/mol
Storage Conditions: Refrigerator
Batch Identification: DAH-XXXIII-35
Purity: 99.1%
Expiration Date: 7/31/2014
Structure:



Common Name: **Compound X**
Synonyms: CSAA130987, CGA142110
CAS Number: 33252-63-0
Molecular Formula: $C_6H_4F_3NO$
Molecular Weight: 163.1 g/mol
Storage Conditions: Refrigerator
Batch Identification: KI-6686/2M
Purity: 99.7%
Expiration Date: 9/30/2013
Structure:



Characterization data for the reference standards are maintained by the Sponsor, Syngenta Crop Protection. The Certificates of Analysis is presented in Appendix 3.

3.2 Test System

The test system for this study was a soil sample collected from Underwood Farm, OH under protocol number TK0002309 (sample ID: RIMV00412-0001).

The soil sample was sent from Syngenta to ADPEN Laboratories, Inc. on 9/12/12 and received on 9/14/12. Upon receipt, the sample was logged into LIMS and assigned a unique laboratory code, which is cross-referenced to the Syngenta sample number on raw data and detailed residue reports. The sample was stored in freezer E-24, which had a temperature range during the course of this study of −27 to −17 °C. Sample extracts were stored in refrigerator E-20 while awaiting LC-MS/MS analysis. The temperature range during the course of this study for this refrigerator was 6–7 °C.

This soil sample was characterized by AGVISE Laboratories of Northwood, North Dakota and reported on 11/9/2010. The GLP soil characterization report is presented in Appendix 4.

3.3 Preparation of Standard Solutions

All standard solutions were prepared and stored as recommended in the method.

3.4 Analytical Procedure

Analytical Method GRM044.03A was independently validated as written. The apparatus and reagents used for the method trial were as outlined in the analytical method with equivalent apparatus or reagents substituted as necessary.

3.4.1 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard at LOQ (1 ppb) and 10× LOQ (10.0 ppb) concentrations as per the method. Fortifications used in this method validation are as follows:

Matrix	Fortification Volume (µL)	Fortification Concentration (ng/mL)	Sample Weight (g)	Final Concentration (ppb)	Replicates
Soil	500	40	20.0 ± 0.1	1.0	5
	500	400	20.0 ± 0.1	10	5

3.4.2 Extraction Procedure

1. Accurately measure and transfer 20 g of each soil sample into a separate 50-mL polypropylene disposable centrifuge tubes.
2. Fortify samples, as necessary.
3. Accurately add 30 mL ACN/Buffer "A" (50:50, v/v) into the sub-sample.
4. Properly cap the vial and shake well at high speed for 25 minutes.
5. Centrifuge samples at 3400 rpm for 10 minutes.
6. Decant supernatant to a clean 50-mL centrifuge tube.
7. Repeat extraction with an additional 20 mL of ACN/Buffer "A" (50:50, v/v).
8. Shake well for 25 minutes using mechanical shaker.
9. Centrifuge samples at 3400 rpm for 10 minutes.
10. Decant the supernatant and combine both supernatant into a 50-mL centrifuge tube.
11. Adjust the final volume to 50 mL with Buffer "A" and vortex the mixture to mix well.
12. Transfer approximately 1 mL of sample to a syringe to filter sample through a 0.2 µm PTFE (polytetrafluoroethylene) syringe filter into a 2-mL glass vial.
13. Transfer a 250-µL aliquot of the resulting filtrate to a 2-mL auto sampler vial containing 750 µL of Buffer "A".
14. Shake the mixture.
15. Vial sample for analysis by LC-MS/MS.

3.4.3 Modifications

No modifications were made to the analytical procedure. Instrument parameter optimizations were made as allowed by the analytical procedure.

3.5 Instrumentation

LC System:	Agilent 1290 Infinity Series
MS Detector:	Agilent 6490 Triple Quadrupole LC-MS/MS

Flow Rate:	0.5 mL/min
Column:	Ascentis Express C_8, 2.7µ, 3.0 × 50.0 mm
Column temperature:	25 °C
Injection Volume:	10 µL
Run Time:	7.5 minutes
Retention Times:	Fluazifop-P-Butyl: 5.37 min; Fluazifop-P Acid: 4.64 min, Compound X: 2.20 min
Mobile Phase A:	0.1% formic acid in HPLC water
Mobile Phase B:	0.1% formic acid in methanol

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.0	0.5	90	10
0.5	0.5	90	10
1.5	0.5	40	60
4.0	0.5	40	60
4.5	0.5	10	90
6.5	0.5	10	90
6.6	0.5	90	10
7.5	0.5	90	10

Mass Spectrometer Conditions

Interface:	ESI
Curtain gas:	14 L/min
Temperature:	100 °C
Capillary (V):	3000
V Charging:	1600
Nebulizer (psi):	45
Sheath gas heater:	300
Sheath gas flow:	12

MRM Conditions	Fluazifop-P-butyl	Fluazifop-P Acid	Compound X
MS1:	384.14	326.06	164.03
MS2:	281.9	253.8	145.9
MS1 Resolution:	Wide	Wide	Wide
MS2 Resolution:	Wide	Wide	Wide
Dwell time:	10	100	100
Frag (V):	380	380	380
Collision Energy (V):	17	12	21
Cell Acc (V):	5	5	5
Polarity:	Positive	Negative	Positive

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by MassHunter Quantitative Analysis (version B.04.01) data handling software. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated using a current Microsoft® Office Excel. Example calculations are presented in Appendix 5.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Prior to conducting the ILV, method control was established. Instrument parameters for the mass spectrometer detector were optimized by infusing standard solutions of the target analytes. The optimized instrument parameters, analyte retention times, instrument detection limits, and linearity were established by injecting a series of calibration standards. The UTC sample and a reagent blank were analyzed to determine if interferences were present near analyte retention times. These analyses verified that the method was in control and the UTC samples were free of interferences.

4.2 Independent Laboratory Results

The method was successfully validated for soil during the first trial at the LOQ (1.0 ppb) and 10× LOQ (10 ppb), using the method as written. Mean recoveries at the LOQ and 10× LOQ were within the acceptable range (70–120%).

TABLE 1 Flow Diagram of the Analytical Procedure

Transfer sample (20 g) to a centrifuge tube
(Fortify recovery sample, if needed)

↓

Add ACN/Buffer "A" (50:50, v/v; 30 mL)

↓

Shake sample at high speed for 25 minutes then centrifuge sample at 3400 RPM for 10 minutes.

↓

Decant supernatant (1) to a clean 50-mL centrifuge tube and repeat extraction with an additional 20 mL of ACN/Buffer "A" (50:50, v/v)

↓

Shake well and centrifuge the samples

↓

Decant the supernatant (2) and combine both supernatants (1) and (2)

↓

Adjust the final volume to 50 mL with Buffer "A"

↓

Vortex the mixture to mix well

↓

Filter approximately 1 mL of the resulting sample through a 0.2 µm PTFE syringe filter

↓

Transfer a 250-µL aliquot of the resulting filtrate to a 2-mL auto sampler vial containing 750 µL of Buffer "A"

↓

Shake the mixture

↓

Transfer samples into HPLC vials

↓

Analysis by LC-MS/MS