2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta Analytical Method GRM044.04A "Fluazifop-P-Butyl – Independent Laboratory Validation of Residue Method (GRM044.04A) for the Determination of Fluazifop-P-Butyl (R154875; PP5), Fluazifop-P Acid (R156172), Compound IV (R150397; CGA181847) and Compound X (R154719; CGA142110) in Water" 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.[1]

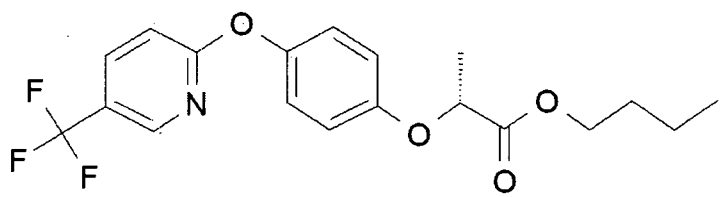
3.0 MATERIALS AND METHODS

3.1 Reference Substances

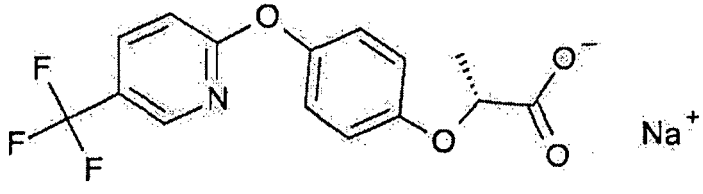
The reference substances were obtained from Syngenta Crop Protection and stored as directed. All fortification and calibration solutions made from the reference substances (analytical standards) were stored according to the method.

The following reference substances were used:

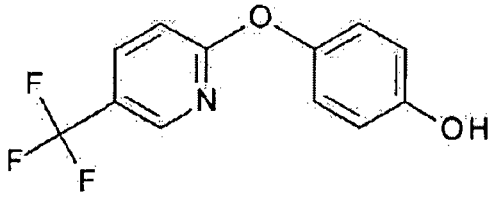
Common Name: **Fluazifop-*p*-butyl**
IUPAC Name: (*R*)-2-[4-(5-trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
CAS Number: 79241-46-6
Molecular Formula: $C_{19}H_{20}F_3NO_4$
Molecular Weight: 383.3 g/mol
Storage Conditions: Refrigerator
Batch Identification: ASJ10030-06
Purity: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



Common Name: **Fluazifop-*p* Acid**
Synonyms: CSCC890014, R156172 (as sodium salt)
IUPAC Name: (*R*)-2-[4-(5-trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
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:

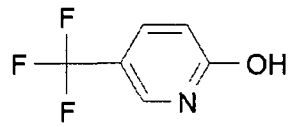


Common Name: **Compound IV**
Synonyms: CSAA169875, CGA 181847
IUPAC Name: 4-(5-trifluoromethyl-pyridin-2-yloxy)-phenol
CAS Number: 69045-85-8
Molecular Formula: $C_{12}H_8F_3NO_2$
Molecular Weight: 255.2 g/mol
Storage Conditions: Refrigerator
Batch Identification: DAH-XXXII-85
Purity: 99.3%
Expiration Date: 05/31/2013
Structure:



Common Name: **Compound X**

Synonyms: CSAA130987, CGA142110
IUPAC Name: 5-trifluoromethyl-pyridin-2-ol
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 are presented in Appendix 3.

3.2 Test System

The test system for this study were untreated control (UTC) surface and ground water samples collected under protocol number TK0015287 (sample IDs: RIMV00512-0001 and RIMV00512-0002).

The water samples were sent from Syngenta to ADPEN Laboratories, Inc. on 9/13/12 and received on 9/14/12. Upon receipt, samples were 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 samples were stored in freezer E24, which had a temperature range during the course of this study of −27 to −17 °C. Sample extracts were stored in refrigerator E20 while awaiting LC-MS/MS analysis. The temperature during the course of this study for this refrigerator was 6 °C.

The water samples were characterized by AGVISE Laboratories of Northwood, North Dakota and reported on 09/04/2012. The GLP water characterization reports are 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 Procedures and Modifications

Analytical Method GRM044.04A 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 water samples were fortified using microliter amounts of the appropriate fortification standard at LOQ (0.10 ppb) and 10× LOQ (1.0 ppb) concentrations as per the method. Fortifications used in this method validation are as follows:

Matrix	Fortification Vol. (µL)	Fortification Conc. (ng/mL)	Sample Wt. (mL)	Final Conc. (ppb)	Replicates
Ground Water	100	10	10.0 ± 0.1	0.10	5
	100	100	10.0 ± 0.1	1.0	5
Surface Water	100	10	10.0 ± 0.1	0.10	5
	100	100	10.0 ± 0.1	1.0	5

3.4.2 Extraction Procedure

1. Accurately measure and transfer 10.0 mL of each water sample into a separate 20-mL disposable glass vials using an automatic pipette.
2. Accurately add 1.0 mL of 2% acetic acid in acetonitrile solution into the sub-sample using an automatic pipette.
3. Properly cap the vial and mix well with a vortex mixer.
4. Transfer approximately 1 mL of the resulting sample into individual HPLC vials and properly cap.
5. 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 S/N USPL001354
Column temperature:	20 °C
Injection Volume:	10 µL
Run Time:	7.5 minutes
Mobile Phase A:	0.1% formic acid in HPLC water
Mobile Phase B:	0.1% formic acid in MeOH

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
Polarity:	Positive
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 IV	Compound X
MS1:	384.14	326.06	256.06	164.03
MS2:	281.9	253.8	93	145.9
MS1 Resolution:	Wide	Wide	Wide	Wide
MS2 Resolution:	Wide	Wide	Wide	Wide
Dwell time:	100	10	100	100
Frag (V):	380	380	380	380
Collision Energy (V):	12	17	16	21
Cell Acc (V):	5	5	5	5
Polarity:	Positive	Negative	Positive	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 recoveries, means, and standard deviations were calculated using a current Microsoft® Office Excel.

TABLE 1 Flow Diagram of the Analytical Procedure

Transfer sample (10.0 mL) to a 20-mL polypropylene disposable tube
(Fortify recovery sample, if needed)

↓

Add 1.0 mL of 2% acetic acid in acetonitrile (v/v)
Note: approximately 10% dilution at this stage

↓

Mix well by agitation (vortex)

↓

Transfer samples into HPLC vials

↓

Analysis by LC-MS/MS