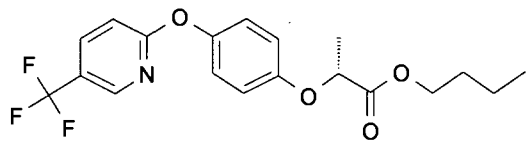
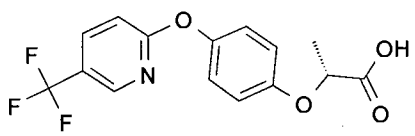
1.0 INTRODUCTION

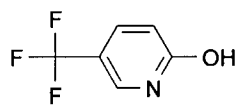
1.1 Scope and chemical structures

This method amendment is to correct typographical errors discovered on the initial method and it supersedes the method GRM044.03A issued on June 17, 2010.

This method is for the residue determination of fluazifop-P-butyl (R154875; PP5) and it's degradates fluazifop-p acid (R156172) and Compound X (R154719; CGA142110) in various types of soil samples. The limit of quantitation (LOQ) has been established at 1.0 ppb for all the targeted analytes. The instrument limit of detection (LOD) is 2.5 picogram (pg), on column, for all the targeted analytes. These LOD's are defined as the lowest concentration of standard injected (on-column injected amount) and used to construct the respective calibration plots. Although the enantiomeric enriched reference materials (PP5 and R156172) are used as reference materials for residue quantification, the chromatographic conditions employed in this method were not designed to resolve the stereoisomerism in racemic mixtures. The chemical structures of the analytes and the corresponding relevant information are listed as follows:

Name/Synonym:	Fluazifop-P-butyl (R154875; PP5)
CAS Name:	Propanoic acid, 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-, butyl ester, (2R)-
CAS Number:	79241-46-6
IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid butyl ester
Structure*:	
Molecular Formula:	$C_{19}H_{20}F_3NO_4$
Molecular Weight:	383.36
Molecular Mass:	383.13

Name/Synonym:	Fluazifop-p Acid (R156172)
CAS Name:	Propanoic acid, 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-, (2R)-
CAS Number:	83066-88-0
IUPAC Name:	(R)-2-[4-(5-Trifluoromethyl-pyridin-2-yloxy)-phenoxy]-propionic acid
Structure*:	
Molecular Formula:	$C_{15}H_{12}F_3NO_4$
Molecular Weight:	327.25
Molecular Mass:	327.07

Name/Synonym:	Compound X (R154719; CGA142110)
CAS Name:	2(1H)-Pyridinone, 5-(trifluoromethyl)-
CAS Number:	33252-63-0
IUPAC Name:	5-Trifluoromethyl-pyridin-2-ol
Structure:	
Molecular Formula:	$C_6H_4F_3NO$
Molecular Weight:	163.09
Molecular Mass:	163.02

* Note: Although the enantiomeric enriched materials are used as reference materials for residue quantification, the chromatographic conditions employed in this method are not designed to resolve the stereoisomerism in racemic mixtures.

1.2 Method Summary

Typically, after thermal equilibration to ambient temperature, a portion of the soil samples (20 g) are extracted two times by shaking with extraction solvent cocktail at room temperature for 20 minutes each. The extracts are combined upon centrifugation and the extraction volume adjusted to 50-mL with buffer solution. Aliquots of the final extracts are filtered with PTFE syringe membrane filter (13mm; 0.45 micron) to remove fine suspension particles prior to final dilution with buffer solution for subsequent LC-MS/MS analysis using electrospray ionization techniques. Residue quantification is carried out using external standard calibrations.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted except in cases where it is noted that no substitution is allowed.

2.2 Reagents

All solvents and other reagents must be of high purity, *e.g.* glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents and analytical standards used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of analytical standard solutions

It is recommended that the following precautions should be taken when weighing the analytical materials:

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth or skin.
4. Wash any contaminated area immediately.

In general, individual primary stock solutions of analytical standards at the 100 µg/mL concentration level is prepared by dissolving 10.0 mg of individual compound into a 100-mL volumetric flask followed by dilution to the mark with HPLC grade acetonitrile. The amount weighed for each compound should be corrected for its respective % purity. If sonication is applied to help dissolution of analytes into solution, allow the solution to return to room temperature before adjusting the final volume.

Alternatively, the appropriate volume of acetonitrile is added to a known amount of standard material using the equation below. The concentration of the analytical standard is corrected for its chemical purity.

$$V(mL)=\frac{wt.(mg)\times P}{C(\mu g/mL)}\times 10^{3}$$

Where "*V*" is the volume of acetonitrile needed; "*wt.*" is the weight, in mg, of the solid analytical standard; "*P*" is the purity, in decimal form, of the analytical standard; "*C*" is the desired concentration of the final solution, in µg/mL; and 10^3 is a conversion factor. In this second case, the standard material is weighed directly into an amber glass storage bottle.

All standard solutions are stored in amber glass bottles in a refrigerator at approximately 4°C to prevent concentration changes due to photodecomposition of the analytes or solvent evaporation. Fresh mixed working standard solutions are typically prepared every three months and fresh individual or mixed stock standard solutions are prepared every six months. In general, the expiration dates of the mixed stock and working standard solutions are not extended beyond the expiration date of the solid standard unless stability considerations or other pertinent information dictate otherwise.

2.3.1 Calibration Standards

Due to possible degradation of the fluazifop-P-butyl (R154875; PP5), this analyte is prepared separately as a lone calibration standard set, while the other two degradates ("Mix-2"; R156172 and CGA142110) are prepared as a mixed calibration standard set. The working calibration standards are prepared from the 100 µg/mL stock standards by transferring 10-mL of the appropriate stock solutions into a 100-mL volumetric flask and diluting to the mark with acetonitrile. These result in separate PP5 and "Mix-2" intermediate working standards at 10 µg/mL concentrations in acetonitrile. Similarly, further dilution of these working standards, individually, with acetonitrile results in a PP5 and a "Mix-2" intermediate working standards at 1.0 µg/mL concentration. The two sets of LC-MS/MS calibration standards are prepared by serial dilutions of the intermediate working standards at 1.0 µg/mL concentration in acetonitrile with 10/90 Acetonitrile/"Buffer A" (v/v) solution (see Appendix 2). Minimum of five concentration levels of calibration solutions, ranging from 0.05 to 10 pg/µL concentrations, are prepared for LC-MS/MS quantification via external standard calibrations.

2.3.2 Fortification Standards

Fortification standards should be prepared, in HPLC grade acetonitrile, at concentrations such that samples are fortified using no more than one thousand micro-liters (1,000 µL) of fortification standard solution. For example, a fortification standard solution at the 1.0 µg/mL concentration could be prepared by taking 10 mL of the working stock standard (10 µg/mL in acetonitrile) into a 100-mL volumetric flask and filling to the mark with HPLC grade acetonitrile. Similarly, a fortification standard solution at the 0.1 µg/mL concentration could be prepared by transferring 10 mL of the fortification standard at 1.0 µg/mL into a 100-mL volumetric flask and filling to the mark with HPLC grade acetonitrile. By using this fortification standard preparation, a 20 g soil sample fortified at 1.0 ppb can be accomplished by fortifying 200 µL of 0.1 µg/mL (*i.e.* ng/µL) fortification solution into the soil sample. Note that the PP5 fortification solutions should be prepared separately as a lone fortification standard solution from the other two targeted analytes ("Mix-2") fortification standard solution due to possible instability of PP5 and to minimize interferences of procedural recoveries among analytes.

2.4 Safety precautions and hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate

MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S. G. Luxon, The Chemical Society, London (Reference 1).

Reagent hazards:

Solvent/Reagent	MeOH	Acetonitrile	Formic Acid	Conc. NH_3
Harmful Vapor	✓	✓	✓	✓
Highly Flammable	✓	✓	✗	✗
Harmful by Skin Absorption	✓	✓	✓	✓
Irritant to Respiratory system & eye	✓	✓	✓	✓
Syngenta Divisional Toxicity Class	SHC-C,S	D,S	SHC-C,S	SHC-C,S
OES Short Term (mg m^{-3})	310	105	N/A	24*
OES Long Term (mg m^{-3})	260	70	9	17*

* Based on NH_3

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

Due to the low detection limit of the method it is important that precautions be taken to avoid cross contamination in the laboratory.

Specifically:

- Wherever possible, disposable glassware/plastic-ware is recommended. If disposable glassware/plasticware has been specified, new glassware/plastic-ware should be used for each batch of samples.
- Each solvent used in the method should be checked to verify that it is free from contamination (if contamination is suspected).
- Existing glassware should be solvent (methanol or acetone) rinsed, after washing and before use in the method.

3.1 Sample Storage and Temperature Re-Equilibration

Soil samples are typically received frozen and stored at freezing temperature (-10°C) until removal for analysis. The sample should be allowed to re-equilibrate to room temperature before removing and transferring a portion for analysis.

3.2 Sample Preparation

a) Weigh representative amounts of soil (20 ± 0.1 g) into separate 50-mL disposable plastic centrifuge tubes. At least one untreated control and two control samples fortified with known amounts of analytes of interest should be analyzed with each sample set, using the same procedure, to verify method performance. No more than 1.0 mL of fortification solution should be added. Allow fortified control samples to equilibrate for at least 20 minutes before proceeding to the extraction.

Note: Due to the chemical instability of PP5 in soil at room temperature, the PP5 fortified samples should be extracted within 20 minutes after fortification to obtain meaningful procedural recoveries.

b) Add 30 mL of 50/50 (v/v) Acetonitrile/"Buffer A" into the centrifuge tube, cap and shake on a mechanical shaker at a speed that visibly agitates the samples for a minimum of 20 minutes. Tubes should be placed in a flat or horizontal orientation.

c) Centrifuge samples at approximately 5000 rpm (or at a speed that visibly separates the solid sample from the supernatant) for about 10 minutes.

d) Decant supernatant to clean, appropriately labeled, 50-mL centrifuge tubes.

Note: With some soils, particularly those with high clay contents, the solution may still be visibly cloudy even after centrifugation. This is normal and will not affect results.

e) Repeat extraction using another 20 mL of 50/50 (v/v) Acetonitrile/"Buffer A" for the remaining solid (soil) in the centrifuge tube from the first extraction at 3.2 (c). Cap and shake by hand or vortex to mix. If shaking cannot break up the compacted soil, use a suitable implement (e.g., a spatula) to facilitate this process. Shake on a mechanical shaker at a speed that visibly agitates the samples for a minimum of 20 minutes. Once again, tubes should be placed in a flat or horizontal orientation

f) Centrifuge samples at 5000 rpm (or at a speed that visibly separates the solid sample from the supernatant) for about 10 minutes.

g) Decant and combine the supernatant into the corresponding 50-mL centrifuge tube containing the first extract from 3.2 (d).

h) Adjust the final volume for the combined supernatants to 50-mL with "Buffer A" and mix well with vigorous shaking or vortexing.

i) Transfer approximately 1-mL of resulting extract from step 3.2(h) using a 3-ml syringe with needle. Remove needle and filter sample through a 0.2 µm PTFE syringe filter (13mm; see Appendix 1) into a 1-mL glass vial.

j) Transfer a 250-µL aliquot of the filtered extract from step 3.2(i) with an automatic pipette into an HPLC vial containing 750-µL of "Buffer A". This results in a four times (4x) dilution of the final extract from step 3.2(i).

Note: The four times (4x) dilution rate is required to maintain proper chromatographic retention of analytes (Compound X in particular) and minimizing possible interferences from the matrices (PP5 in particular).

Higher dilution ratio and re-analysis may be required when (1) samples contain residues greater than 100 ppb and/or (2) earlier analytical runs indicated possible interferences which may affect residue determinations.

k) Cap each HPLC vial, mix well with a vortex, and subject the sample to LC-MS/MS for residue analysis.

3.3 Fortification

Soil samples can be fortified for procedural recovery purposes by judicious choice of working solution concentration and volume. For example, the addition of 200µL of a 0.10 µg/mL (ng/µL) fortification standard solution to a 20 ±0.1 grams portion of soil sample produces a 1.0 ppb fortification sample. The fortification levels used in each set of analyses can vary, but should always include one recovery sample at the LOQ level. Furthermore, separate fortification for fluazifop-P-butyl and "Mix-2" is required for recovery evaluation.

3.4 Time Required for Analysis

In the method validation, a typical validation set consisted of a batch of 33 samples from each type of soil. A typical analytical sequence included these 33 samples and the required calibration standards. One skilled analyst can complete the sample preparation of one set of 33 samples within approximately 8 working hours. The analytical sequence was typically performed overnight on a LC-MS/MS system.

3.5 Method Stopping Points

Procedural stopping is typically not required due to the simplistic operational procedures of the method. It has been demonstrated, during the method development, that the soil extracts or the excess portions of the final soil extracts can be stored under refrigeration up to one week for further processing or analysis without re-extraction of the soil. In this case, the sample extracts should be stored in sealed containers at refrigerated temperatures when the analysis cannot be completed in a single working day. However, it is generally not recommended unless deemed necessary when instrumental difficulties are encountered during the time of analysis. In this case, the spiked samples and calibration standards should be stored in sealed vials at refrigerated temperatures when the analyses cannot be completed in a single working day.

3.6 Preparation of Calibration Standards for LC-MS/MS

As outlined in Section 2.3.1, standards for multi-point calibration should be prepared in 10/90 (v/v) Acetonitrile/"Buffer A" solution. In general, it is recommended that a minimum of five levels of calibration standards be used for calibration plot establishment. In the method validation, the following concentration levels of standards were prepared for calibration plots: 0.05 pg/µL, 0.10 pg/µL, 0.20 pg/µL, 0.50 pg/µL, 1.0 pg/µL, 2.0 pg/µL, 5.0 pg/µL, and 10 pg/µL.

All the LC calibration standards and fortification standards should be stored in amber glass bottles under refrigeration conditions (approximately 4°C). For the "PP5" standards, an expiration of one month for calibration standards and two months for fortification standards is recommended. For the "Mix-2" standards, an expiration of three months for calibration standards and fortification standards is recommended. The expiration of calibration and

fortification standards should not be extended unless additional study data are generated that show a longer expiration date is appropriate.

4.0 FINAL DETERMINATION

A Thermo Electron TSQ Quantum Ultra mass spectrometer was used to establish and validate the method. The system is controlled and data is processed by Thermo Electron Xcalibur™ Software. Other instruments may also be used, however optimization may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum instrument operation.

Following are the typical instrumental parameters applied for this method during method validation. The analyst should make necessary adjustments and tuning to these parameters to obtain optimum operational conditions based on the actual instrument used for the specific study.

4.1 LC System Description and Operating Conditions

LC Instrumentation:

The Surveyor Plus LC system consists of an analytical pump unit (a quaternary solvent system) and an autosampler. The solvent degasser, column oven, and sample tray temperature control are integral parts of the LC system. The system is controlled and data processed by Thermo Electron Xcalibur™ Software.

LC Operating Conditions:

Injection Volume: 50 µL
Sample Compartment Temp.: refrigerated at 15°C (recommended)
Column Temperature: 25°C (recommended)
Column: Ascentis Express C8, 50 x 3.0 mm, 2.7 µm (Supelco Cat. no. 53848-U)
Column filter: ColumnSaver (MAC-MOD Catalog No. MMCS210)
Mobile Phase A: 0.1% formic acid in HPLC grade water
Mobile Phase B: 0.1% formic acid in HPLC grade methanol

Step	Time (min)	%A	%B	Flow Rate (mL/min)	Gradient
0	0.0	90	10	0.5	---
1	0.5	90	10	0.5	---
2	1.5	40	60	0.5	linear
3	4.0	40	60	0.5	---
4	4.5	10	90	0.5	linear
5	6.5	10	90	0.5	---
6	6.6	90	10	0.5	linear
7	7.5	90	10	0.5	---

The typical retention times for the analytes are listed in Section 4.2 when using this instrumentation and conditions. The retention time may vary depending upon chromatographic conditions and systems.

Note: To help minimizing instrument contamination, a timed event controlled switching valve may be used to divert the LC stream to waste during periods of no data collection.

4.2 Mass Spectrometer Conditions

A Thermo Electron TSQ Quantum Ultra mass spectrometer was used to establish and validate the method. The system is controlled and data processed by Thermo Electron Xcalibur™ Software. Electrospray ionization (ESI) source with positive and negative ion detection modes are applied for analysis.

The following are the typical instrumental parameters applied for this method during method validation. Alternative instrument with comparable sensitivity and performance criteria can be used for this method. The analyst should make necessary adjustments and tuning of the instrument parameters to obtain optimum operational conditions based on the actual instrument used for the specific study.

Ion Source Parameters:

	Positive Mode	Negative Mode
Spray Voltage (V)	3500	2500
Vaporization Temperature (°C)	350	350
Sheath Gas Pressure (psi)	45	45
Ion Sweep Gas Pressure (psi)	5.0	5.0
Aux Gas Pressure (psi)	40	40
Capillary Temperature (°C)	300	300
Tube Lens Offset	50 - 110	(-50) – (-110)
Skimmer Offset (V)	0	0
Collision Pressure (mTorr)	1.0	1.0

Note: The mass spectrometer tuning parameters shown here are for reference only. The analyst should consult with instrument operation manual to obtain optimum conditions for all the analytes prior to residue analysis.

MRM (SRM) Operating Parameters:

MS/MS Transitions

Analyte	MS/MS Transition*	Scan Width	Dwell (sec.)	CE (Volts)	Q1 PW	Q3 PW	RT (min.)
Compound X (R154719; CGA142110)	Positive mode						
Quantification	164.05 → 146.00	0.01	0.02	25	0.7	0.7	2.82
Confirmation	164.06 → 75.00	0.01	0.02	40	0.7	0.7	2.82
Fluazifop-P Acid (R156172)**	Negative mode						
Quantification	326.06 → 254.00	0.01	0.02	16	0.7	0.7	5.32
Confirmation	326.07 → 226.10	0.01	0.02	25	0.7	0.7	5.32
Fluazifop-P-Butyl (R154875; PP5)**	Positive mode						
Quantification	384.15 → 328.00	0.01	0.02	18	0.7	0.7	6.18
Confirmation	384.14 → 282.00	0.01	0.02	22	0.7	0.7	6.18

Data collection windows:
2.0 – 4.9 minutes is positive; 4.9 – 5.7 minutes is negative and 5.7 – 6.6 minutes is positive.

* The specified mass difference of 0.01 amu for precursor ions in quantification and confirmation detections is required for channel separation of signals on the Thermo Electron TSQ Quantum Ultra mass spectrometer with Xcalibur™ software. The MS/MS transitions listed were the most sensitive and stable transitions for the corresponding analytes based on the optimal tuning parameters obtained prior to method validation with Thermo Electron TSQ Quantum Ultra instrument. Alternative MS/MS transitions may be used if different comparable instrument is applied or encounter interferences. Analysts should consult with instrument operation manuals for the specifics and adjustments when using instruments from different manufacturers to obtain optimum results.

** Although the enantiomeric enriched materials are used as reference materials for residue quantification, the chromatographic conditions employed in this method are not designed to resolve the stereoisomers in racemic mixtures.

5.0 CALCULATION OF RESULTS

Determination of Residues in Samples:

Analyze the samples prepared as described in Section 3.2 on the LC-MS/MS system along with a selected range of calibration standards. Calibrate the instrument by intermittently injecting at least five (or more) concentration levels of the standard solutions and generate a calibration curve for the analyte using proper regression parameters (*e.g.* linear or quadratic regression with 1/X weighing) with external standard calibration. Forcing the calibration curve through the origin is not recommended. The data system (*e.g.* Xcalibur™) uses the calibration plot and the respective peak responses (*e.g.*, area or height) to calculate the amount of analyte in a sample. If the analyte response in the sample exceeds 10% of the

response for the highest concentration standard injected, the sample should be diluted with 10/90 (v/v) acetonitrile/"Buffer A" solution and re-analyzed.

Procedural Recoveries:

The procedural recovery data for each set of sample analyses must fall within EPA's acceptance criteria of mean recoveries from 70 to 120% and standard deviations of ≤ 20%. Recovery samples are corrected for control values when detected.

Calculations:

Calculations may be performed by computer program (preferred) or manually as shown below.

Calculate the analyte concentration (in ppb) for field-incurred residues using the equation:

$$RES\ (ppb) = \frac{Analyte\ found\ (pg)}{SWI\ (mg)}$$

where *RES* is the residue value in ppb, *Analyte Found (pg)* is calculated from the standard calibration curve, and *SWI* is the sample weight injected in milligrams (*mg*).

The amount (*mg*) of sample weight injected (*SWI*) can be calculated using the equation:

$$SWI\ (mg) = \frac{FW\ (g) \times IV\ (\mu L)}{FV(mL)}$$

where *FW* = final sample weight (g), *IV* = LC injection volume (µL) and *FV* = final volume in which sample is dissolved (mL).

The final sample weight (*FW*) is calculated by the equation:

$$FW\ (g) = \left[\frac{SWE\ (g) \times A1\ (mL)}{EV\ (mL) + \{SWE\ (g) \times M\ (\%)/100\}}\right] x \left[\frac{A2\ (mL)}{INV\ (mL)}\right]$$

where *FW* = final weight (g), *SWE* = sample weight extracted (g), *A1* = aliquot 1 volume (mL), *EV* = total extraction solvent volume (mL), *M* = sample moisture in percent, *A2* = aliquot 2 volume (mL), if needed, *INV* = interim volume (mL) is the total volume from which the second aliquot is taken.

NOTES: For method performance (recovery) samples, the *M*% (moisture) value is set to zero since the fortifications are based upon their wet weights. If no sample dilutions are performed, the second term in the equation (i.e., *A2/INV*) is equal to one.

Corrections may be made to the residue value (*RES*) calculated above. At the discretion of the study director, this value may be corrected to account for the average recovery and/or sample moisture.

The recovery factor, expressed as a percentage (*R%*), is calculated using the following equation.

$$R\% = \frac{RES\ fortified\ (ppb) - RES\ control\ (ppb)}{ppb\ \ analyte\ \ added} \times 100$$

To correct a residue value to its dry weight value, the following equation may be used:

$$SDW\ (ppb) = \left[\frac{CR\ (ppb)}{\frac{(100 - M(\%))}{100}} \right]$$

where *SDW* = soil dry weight residue (ppb), *CR* = corrected soil residue, and *M* = soil moisture (%). For study samples, soil moistures should be determined following the appropriate SOP.

The recovery corrected soil residue can be determined by the equation:

$$CR\ (ppb) = \left[\frac{RES\ (ppb)}{AR\ (\%)} \right] x\ 100$$

where *CR* = recovery corrected residue (ppb), *RES* = residue found (ppb) and *AR* = average recovery (%).

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

6.0 INTERFERENCES AND CONFIRMATION

Due to the highly selective nature of the detection technique using tandem mass spectrometry, interference arising from the sample matrix has not been observed in the validation study. Although residue determination by LC-MS/MS is considered to be highly specific, a secondary MS/MS transition for the specific analyte can be acquired for confirmatory purposes when needed.

It is recommended that each batch of solvent or reagents be checked for potential contamination prior to use. This method uses disposable labware, where possible. All reusable glassware should be detergent washed then rinsed with HPLC grade methanol or acetone and thoroughly dried prior to use.

7.0 MODIFICATIONS AND POTENTIAL PROBLEMS

It is possible that contaminants from chemicals, solvents, glassware, etc. may interfere with the analysis and give a false positive result with confirmatory measures in place. It is recommended that reagent blank samples be included in a sample set if contamination is suspected. During the method development, minor residual carryover of fluazifop-P-butyl

(PP5) was observed immediately after high level standards or samples with high concentration of residues (e.g. 20 pg/µL concentrations). The typical amounts of carryover were estimated to be less than 50% of the method LOD at concentration of 10 pg/µL (i.e. < 1.25 pg; on column), therefore, this carryover will not affect the accuracy of residue determination of this method. If carryover issues are suspected, injection of 10/90 acetonitrile/"Buffer A" (v/v) solution immediate after the high level calibration standard or high residue containing samples is recommended to minimize the effect. In general, the effective method LOQ should be adjusted and reported accordingly if the carryover issue cannot be resolved.

The quality of the calibration plot can deteriorate if the ESI source becomes too dirty. Thus, inspection of each calibration plot needs to be performed in order to maintain accurate and reliable quantification of each set of samples. In general, calibration plots exhibiting good regression analysis characteristics with $R^2 \geq 0.99$ is considered acceptable. Furthermore, the chromatographic conditions employed were not designed to resolve the stereoisomers in racemic mixtures. Any modifications to this method must be documented in the study raw data.

APPENDIX 1 Apparatus

General laboratory glassware (*e.g.* beakers, graduated cylinders, flat bottom flasks, round bottom flasks, pipette bulbs, etc.) are available from a general laboratory supply company.

1. Balance, analytical (Sartorius Model R160P). Electronic display of 0.01 mg, for weighing in preparation of the stock standard solutions.
2. Balance, laboratory (Mettler Model PB3002-S). Electronic display of 0.01 g, for weighing soil samples.
3. Mechanical reciprocating shaker, IKA Labortechnik Model KS501.
4. Refrigerated Centrifuge, Du Pond Instruments, Model Sorvall® RC-5B.
5. pH Meter, Denver Instruments, pH/ISE meter, Model 225.
6. Fisher Variable-Speed Touch Mixer, Fisher Scientific, Catalog No. 12-812
7. Volumetric Pipettes, glass, Class A certified, assorted volumes. (These pipettes should be used for sample fortification and standard solution preparation)
8. Bottles, amber glass Boston round, 4 oz., with Polyseal-lined cap, Fisher Scientific Catalog. No. 02-911-895
9. Disposable centrifuge tubes, polypropylene, 50-mL, graduated with plastic screw cap, Fisher Scientific Catalog No. 05-538-68
10. Brinkmann Eppendorf Pipettor Tips, 200 µL tip, Fisher Scientific Catalog No. 022491334
11. Brinkmann Eppendorf Pipettor Tips, 1000 µL tip, Fisher Scientific Catalog No. 022491351
12. Brinkmann Eppendorf Pipettor Tips, 5 mL, Fisher Scientific Catalog No. 022491385
13. Brinkmann Eppendorf Pipettor Tips, 10 mL, Fisher Scientific Catalog No. 05-403-119
14. Brinkmann Eppendorf 2100 Research Series Pipettor, range 20 - 200 µL, Fisher Scientific Catalog No. 05-402-89
15. Brinkmann Eppendorf 2100 Research Series Pipettor, range 100 - 1000 µL, Fisher Scientific Catalog No. 05-402-90
16. Brinkmann Eppendorf 2100 Research Series Pipettor, range 500 - 5000 µL, Fisher Scientific Catalog No. 05-402-91
17. Brinkmann Eppendorf 2100 Research Series Pipettor, range 1 - 10 mL, Fisher Scientific Catalog No. 05-403-121
18. Disposable Pasteur Pipettes, 146 mm, Fisher Scientific, Catalog No. 22-230-482
19. Disposable syringe with needle, 3-mL, Fisher Scientific, Catalog No. 309579
20. PTFE syringe filter, 13mm diameter, 0.2 µm pore, Fisher Scientific, Catalog No. SLFG013NL

21. Disposable 1-mL clear glass vials, VWR, Catalog no. 66015-702
22. Auto-sampler vials, National Scientific C4011-6W, Fisher Scientific Catalog No. 03-395H
23. Auto-sampler vial enclosures, National Scientific C4011-55R , Fisher Scientific Catalog No. 03-396AA
24. HPLC column filter, MAC-MOD Analytical Inc., P/N MMCS210
25. HPLC column: Ascentis Express C8, 50 x 3.0 mm, 2.7 µm, Supelco Cat. no. 53848-U

Note: Unless otherwise noted, other manufacturers equivalents of the items listed above can be used; however, the use of the substitutes must be demonstrated by obtaining acceptable procedural recoveries. In general, Class A glass volumetric flasks and pipettes were utilized for standard solution preparation and are not individually listed.

APPENDIX 2 Reagents

1. Water, HPLC grade, Fisher Scientific, Catalog No. W5SK-4
2. Methanol, HPLC grade, Fisher Scientific, Catalog No. A452SK-4
3. Acetonitrile, HPLC grade, Fisher Scientific, Catalog No. A998SK-4
4. Glacial Acetic Acid, HPLC grade, Fisher Scientific, Catalog No. A38-500
5. Ammonium Hydroxide, Certified ACS Plus, Fisher Scientific, Catalog No. A669-500
6. Formic Acid, 88%, Certified ACS, Fisher Scientific, Catalog No. A118P-500

   Note: Equivalent reagents obtained from other manufacturers can be used instead of the reagents described above; however, it is important to verify the quality of the solvents to insure there are no interfering contaminants.

7. "0.1%" Formic Acid in HPLC H_2O; prepared by mixing 1.0 mL of formic acid with 1000 mL of HPLC grade Water.
8. "0.1%" Formic Acid in HPLC Methanol; prepared by mixing 1.0 mL of formic acid with 1000 mL of HPLC grade methanol.
9. "Buffer A"; "10mM" ammonium acetate buffer at pH 5.5, prepared by mixing 1-L of HPLC water with 0.57-mL of glacial acetic acid. Using a calibrated pH meter, adjusted the pH to 5.5 using dropwise additions of a 10% ammonium hydroxide (NH_4OH) solution.
10. "10%" Ammonium Hydroxide; prepared by mixing 10-mL Ammonium Hydroxide with 90-mL HPLC grade Water.
11. Acetonitrile/"Buffer A", 10/90 (v/v); prepared by mixing 100 mL of HPLC grade acetonitrile and 900 mL of "Buffer A".
12. Acetonitrile/"Buffer A", 50/50 (v/v); prepared by mixing 500 mL of HPLC grade acetonitrile and 500 mL of "Buffer A".
13. The reference standards used in this method were supplied by the Analytical and Product Chemistry Department or Chemical Synthesis Group of Syngenta Crop Protection, Inc.

Fluazifop-P Butyl (PP5, R154875)	CAS RN: 79241-46-6 CAS Name: Propanoic acid, 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-, butyl ester, (2R)-
Fluazifop-P Acid (R156172)	CAS RN: 83066-88-0 CAS Name: Propanoic acid, 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-, (2R)-
CGA142110 (Compound X)	CAS RN: 33252-63-0 CAS Name: 2(1H)-Pyridinone, 5-(trifluoromethyl)-