2.0 INTRODUCTION

Described in this report is the independent laboratory validation of Syngenta Analytical Method GRM060.08A (Reference 1) as performed by PASC.

This study was designed to satisfy guideline requirements described in EPA 850.6100 (2012) (Reference 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The residue analytical method is deemed suitable for the determination of Flumetralin in soil.

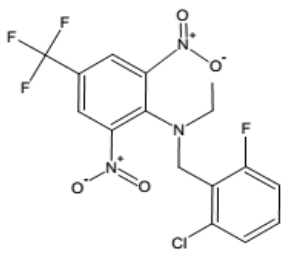
Soil sample contents are extracted with methanol:water (80/20 v/v). An aliquot of sample is partitoned into hexane:toluene (50/50 v/v) and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for analysis.

The validated limit of quantitation of method GRM060.08A is 0.01 mg/kg in soil.

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

The test/reference substance was obtained from Syngenta Crop Protection, LLC. The following test/reference substance was used:

Compound Structure	
Syngenta Code:	CGA41065
Common Name:	Flumetralin
CAS Name:	N-ethyl-N-(2-chloro-6-florobenzyl)-4-triflurомethyl-2,6-dinitroaniline
Batch ID:	410533
Molecular Weight:	421.7 g/mol
Storage Conditions:	Refrigerate < 30ºC
Purity:	99.9%±0.5%
Expiration Date:	End of March 2022

Characterization data for the test/reference standard are maintained by Syngenta Crop Protection, LLC. The Certificate of Analysis is included in Appendix 2.

The test/reference substance (Flumetralin) used in this study was procured from Syngenta Crop Protection, LLC located at the Greensboro facility. All solutions made from Flumetralin standard were stored according to Section 2 of the method.

3.2 Test System

The test system evaluated for this ILV was Soil.

3.3 Equipment and Reagents

The equipment and reagents used for the ILV were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. All solvents and other reagents must be of high purity, e. g. glass distilled/HPLC grade solvents and analytical grade reagents.

3.4 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in Section 2 of the method (Reference 1).

3.4.1 Stock Standard

One 100 µg/mL stock solution for flumetralin was prepared in acetone.

3.4.2 Fortification Standard

Sample fortification solutions containing flumetralin were prepared by serial dilution in acetone from the stock solution. The following solutions were prepared: 10.0 µg/mL, 1.0 µg/mL and 0.1 µg/mL for fortification purposes.

3.4.3 Calibration Standard

Calibration standards were prepared by serially diluting stock standards using hexane:toluene (50/50 v/v). Using equivalent GC-MS instrumentation described in the method, the following concentration range of standards (0.25 pg/µL, 0.5 pg/µL, 10 pg/mL, 2.5 pg/µL, 5 pg/µL, and 10 pg/µL) were prepared and used to construct the calibration plots.

3.5 Analytical Procedures and Modifications

Analytical Method GRM060.08A (Reference 1) was successfully validated by an independent laboratory as written using the procedures and instrumentation recommended by the method. Soil contents are extracted with methanol:water (80/20 v/v). An aliquot of sample is partitioned into hexane:toluene (50/50 v/v) and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for m/z 421, 423, and 391 for analysis. The limit of quantitation of Analytical Method GRM060.08A (Reference 1) is 0.01 mg/kg in soil.

3.5.1 Modifications

Syngenta Analytical Method GRM060.08A (Reference 1) was followed as written.

3.5.2 Fortifications

Untreated control soil samples were fortified using 100 µL of known amounts of flumetralin to LOQ and 10X LOQ concentration levels as per the method. See Table 2 for detailed fortification levels. Fortifications used in this ILV are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/mL)	Replicates
LOQ	100	1	5
10X LOQ	100	10	5

3.5.3 Method Summary

As per Analytical Method GRM060.08A, soil contents are extracted with methanol:water (80/20 v/v). An aliquot of sample is partitioned into hexane:toluene (50/50 v/v) and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for m/z 421, 423, and 391 for analysis.

3.5.4 Limit of Detection and Limit of Quantitation

The limit of detection (LOD) of the method is defined as the lowest analyte amount injected on column detectable above the mean amplitude of the background noise at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. The limit of detection using the instrumentation for this validation was estimated to be 0.5 pg on column. Note that the LOD may vary between runs and from instrument to instrument.

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated with a mean recovery of 70 - 110% and a relative standard deviation of ≤ 20% has been obtained. A limit of quantitation (LOQ) of 0.01 mg/kg in soil was successfully validated in this study.

3.5.5 Detector Linearity

The linearity of the detector response was assessed using a calibration curve generated with each analysis sequence injected. It was shown that the GC-MS detector response for flumetralin has a correlation coefficient ≥ 0.995 in the range from 0.25 pg/uL to 10.0 pg/uL or 0.5 pg to 20.0 pg on column when using a 2 µL injection volume.

Representative plots of the detector responses versus the analyte concentration for all calibration points are presented in the Figures Section.

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by "Chemstation Software version G1732BA, B.02.00.589". 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 the correlation coefficients (R^2) for the calibration curves for each analytical set was ≥ 0.995. 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 Microsoft Office Excel (2007).

APPENDIX 3 GC-MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis. Perform instrument auto tune of compound specific tune using specific calibration masses.

Tuning Instrument for flumetralin

Determine ionization mode and detection (EI or CI).

Perform scan of expected masses. Determine target ion and qualifier ions. Target plus two qualifiers above 100 amu are recommended.

For flumetralin, in negative ion chemical ionization mode, the deprotonated molecular ion generated is selected (*m/z* 421) as the target ion. The two most sensitive qualifier ions (*m/z* 423 and *m/z* 391) are then selected for confirmation.

Daughter Ion *m/z*	Structure
423	^{37}Cl isotope
391	Loss of H_2O

APPENDIX 4 Method Flow Chart