2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta Analytical Method GRM023.05A "SYN524464 - Analytical Method for the Determination of Residues of the Metabolites CSCD465008 and CSAA798670 in Soil. Final Determination by 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.

The residue analytical method is suitable for the determination of CSCD465008 and CSAA798670 in soil. A brief summary of the method is as follows.

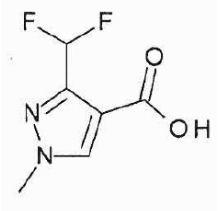
Soil samples were weighed (10 g) and formic acid in ultra-pure water (0.2%, v/v) was added. The sample was shaken for 1 hour at room temperature and centrifuged. A 20-mL aliquot (4 g) was acidified and taken through a solid phase extraction procedure, using Oasis HLB SPE cartridges and acetonitrile/ultra-pure water (50:50, v/v). The eluate was evaporated to remove acetonitrile and diluted with ultra-pure water. Final determination by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS) was utilized in this study. The limit of quantitation of the method is 0.50 ppb.

3.0 MATERIALS AND METHODS

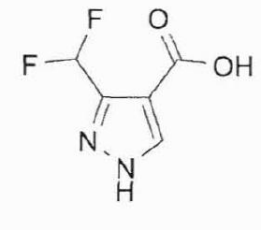
3.1 Test/Reference Substance

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

Alternative Number: NOA 449410
Common Name: CSAA798670
IUPAC Name: 3-(Difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxylic acid
CAS Number: 176969-34-9
Molecular Weight: 176.1 g/mol
Standard Reference: DAH-XXXII-79
Storage Conditions: Refrigerator
Purity: 97.3%
Expiration Date: 01/31/2013
Structure:



Alternate Name: R958945
Common Name: CSCD465008
IUPAC Name: 3-(Difluoromethyl)-1*H*-pyrazole-4-carboxylic acid
CAS Number: Not in registry
Molecular Weight: 162.1 g/mol
Standard Reference: DAH-XXXIV-33
Storage Conditions: Refrigerator
Purity: 90.1%
Expiration Date: 03/31/2014
Structure:



Characterization data for the test/reference standard are maintained by the Sponsor, Syngenta Crop Protection. The Certificate of Analysis is included in Appendix 3.

The test/reference substance (analytical standard) used in this study was procured from the Sponsor and stored as directed. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was sandy loam, because it is representative of the matrix for which the method was designed. The control sample used in this study was characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0002309. GLP characterization results are presented in Table 1 and summarized below.

Sample ID	pH	Calcium (ppm)	Magnesium (ppm)
10-20-10 Ottawa MI 0–6″	7.2	1115	153

Processed samples were sent from Syngenta to ADPEN Laboratories, Inc. on 8/16/12 and received on 8/17/12 (All samples were processed before receipt at ADPEN). Upon receipt, samples were logged in and stored in freezer E24, which had a temperature range during the course of this study of −21 to −27 °C. Prior to analysis, the samples were sub-sampled and unique laboratory codes were assigned to each sub-sample and are cross-referenced on each page of the detailed residue reports to the Syngenta sample number. Sample extracts were stored in refrigerator E54 while awaiting LC-MS/MS analysis. The temperature range during the course of this study for this refrigerator was 6–7 °C.

The control sample was checked for contamination prior to use in this ILV study by employing the same extraction and detection method as described in Syngenta Method GRM023.05A.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method.

3.3.1 Equipment

HPLC system: Agilent Technologies, 1290 Infinity.
LC-MS/MS system: Agilent Technologies, 6490 Triple Quadrupole LC-MS/MS.

3.3.2 Reagents

Reagents used were as described in the method.

3.3.3 Preparation of Reagents

Reagents prepared as described in the method.

3.4 Preparation of Standard Solutions

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

3.5 Analytical Procedures and Modifications

Analytical Method GRM023.05A was independently validated as written.

3.5.1 Modifications

No modifications to the method were made. Equipment was substituted to an Agilent 6490 LC-MS/MS of at least equivalent performance specifications. During the method establishment phase, the LC-MS/MS instrument was optimized to best primary transitions.

3.5.2 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard at LOQ (0.0005 ppb) and 10× LOQ (0.005 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. (g)	Final Conc. (ppb)	Replicates
Sandy Loam	500	10	10.0 ± 0.1	0.50	5
	500	100	10.0 ± 0.1	5.0	5

3.5.3 Extraction Procedure

1. Weigh 10 g of soil into a 250-mL polypropylene disposable centrifuge tube.
2. Fortify samples, if needed.
3. Add 50 mL of formic acid in HPLC water (0.2%, v/v). Shake sample at high speed for 60 minutes.
4. Centrifuge sample at 3500 rpm for 5 minutes.
5. Transfer a 20-mL aliquot into a polypropylene centrifuge tube and acidify with concentrated hydrochloric acid to a pH <2. Check pH with a suitable indicator strip.
6. Place Water Oasis HLB (60 mg, 3 mL) cartridge on a vacuum manifold and condition with 2 mL high purity MeOH. Draw under gravity, followed by 2 mL of HPLC water. Draw under gravity to top of frit discarding eluates. Do not allow column to become dry.
7. With a connector, attach a 20-mL column reservoir to the cartridges and add extract from Step 5. Draw sample through the column at a rate of 1 mL/min, discarding eluates. Do not allow cartridge to go dry.
8. When extract reaches the top of the frit, remove column reservoir and add 2 mL HPLC water to SPE cartridge. Remove excess water under high vacuum for a few seconds, discarding eluates.
9. Eluate analytes under gravity with 2 mL of ACN/HPLC water (50:50, v/v) collecting in a 15-mL centrifuge tube. After elution is completed apply positive pressure to collect all eluate.
10. Remove all ACN under nitrogen at 30 °C to a final volume of ~0.8 mL.
11. Adjust final volume to 1 mL with HPLC water. Vortex sample to mix well.
12. Vial sample for analysis by LC-MS/MS.

3.6 Instrumentation

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

Flow Rate:	0.5 mL/min
Column:	Develosil RP-Aq; 3 µm, 150 × 3.0 mm
Column temperature:	40 °C
Injection Volume:	10 µL
Run Time:	7 minutes
Retention Times:	CSAA798670: 4.04 min; CSCD465008: 2.864 min
Mobile Phase A:	0.1% acetic acid in HPLC water
Mobile Phase B:	0.1% acetic acid in ACN

Mass Spectrometer Conditions

Interface:	ESI
Polarity:	Negative
Curtain gas:	14 L/min
Temperature:	150 °C
Capillary (V):	3000
V Charging:	1500
Nebulizer (psi):	45
Sheath gas heater:	300
Sheath gas flow:	12

MRM Conditions	**CSAA798670**	**CSCD465008**
MS1:	175.03	161.01
MS2:	130.8	140.8
MS1 Resolution:	Wide	Wide
MS2 Resolution:	Wide	Wide
Dwell time:	100	100
Frag (V):	380	380
Collision Energy (V):	9	5
Cell Acc (V):	5	5
Polarity:	Negative	Negative

3.7 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. Recovery results were computed for each sample.

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