2.0 INTRODUCTION

This final report describes the independent laboratory validation (ILV) of Syngenta Analytical Method GRM010.04A "Cyprodinil - Analytical Method (GRM010.04A) for Determination of CGA219417 and CGA249287 in Water by LC-MS/MS" (Reference 1) as performed by ADPEN Laboratories, Inc. (ADPEN). The analytical method is presented in Appendix 2.

This study was designed to satisfy harmonized guideline requirements described in EPA Guideline OCSPP 850.6100 (2012) (Reference 2), EC SANCO/3029/99 Rev 4 (2000) (Reference 3), and EC SANCO/825/00 Rev 8.1 (2010) (Reference 4). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (Reference 5).

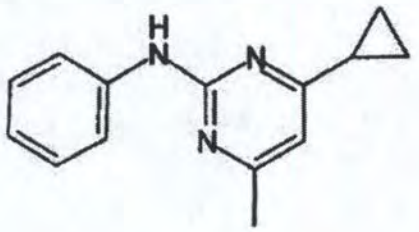
The method consists of vigorously shaking an aliquot of the water sample for 10 seconds and vortexing to mix well. An aliquot is then taken for determination by LC-MS/MS.

3.0 MATERIALS AND METHODS

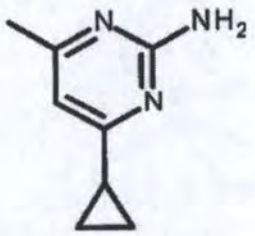
3.1 Test/Reference Substance

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

Common Name:	**Cyprodinil**
Code Name:	**CGA219417**
IUPAC Name:	(4-Cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine
CAS Number:	121552-61-2
Molecular Formula:	$C_{14}H_{15}N_3$
Molecular Weight:	225 g/mol
Batch Identification:	AMS 452/3
Purity:	99.9%
Expiration Date:	End of July 2018
Storage Conditions:	< 30 °C
Source:	Syngenta Production Chemistry



Common Name:	**None**
Code Name:	**CGA249287**
IUPAC Name:	4-Cyclopropyl-6-methyl-pyridin-2-ylamine
CAS Number:	92238-61-4
Molecular Formula:	$C_8H_{11}N_3$
Molecular Weight:	149 g/mol
Lot Number:	GAN-XXXII-31-1
Purity:	99.4%
Expiration Date:	May 31, 2016
Storage Conditions:	Refrigerator
Source:	Syngenta Product Safety Greensboro-Logistics and Support



The test/reference substances (analytical standards) used in this study were procured from the Sponsor and stored as directed. Characterization data for the test/reference standards are maintained by the Sponsor, Syngenta Crop Protection, LLC. The Certificates of Analysis are included in Appendix 3.

3.2 Test System

The test systems evaluated in this study were surface and ground water. Control samples used in this study were characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0165283. GLP characterization results are presented in Appendix 4 and summarized below.

Sample ID	Water Type	Sample Description
RIMV00113-0001	Surface Water	RIMV00312-0001 Control Water Sample
RIMV00113-0002	Ground Water	RIMV00312-0002 Control Water Sample

Control water samples utilized for this study from Syngenta Study Number TK0165283 were sent from Syngenta to ADPEN on August 19, 2013 and received on August 20, 2013. Upon receipt, the samples were logged in and stored in freezer E-23, which had an average temperature during the course of this study of −17 °C. Prior to analysis, the sample was sub-sampled and unique laboratory codes were assigned to each sub-sample and are cross-referenced on each page of the detailed residue reports in Appendix 6 to the Syngenta sample number. Sample extracts were stored in refrigerator E-20 while awaiting LC-MS/MS analysis. The average temperature during the course of this study for this refrigerator was 6 °C.

The control samples were checked for contamination prior to use in this ILV study by employing the same extraction and detection method as described in Syngenta Method GRM010.04A.

3.3 Apparatus

The equipment and apparatus used for the independent laboratory validation were as outlined in the method. Identical or equivalent equipment was used, as permitted by the method.

3.4 Reagents

Reagent	Description	Supplier
Acetonitrile	HPLC grade	EMD
Optima Water	HPLC grade	EMD
Optima Methanol	HPLC grade	EMD
Ammonium Acetate	Analytical Reagent	Sigma Aldrich
Cyprodinil analytical standard	GLP certified	Syngenta
CGA249287 analytical standard	GLP certified	Syngenta

3.4.1 Preparation of Reagents

Reagents were prepared as described in the method.

3.4.2 Preparation of Stock Standard Solutions

Approximately 5 mg of each analytical standard was weighed into a 25-mL volumetric flask (or 20-mL volumetric flask). The volume was brought up to the mark to prepare a 0.1 mg/mL and 0.2 mg/mL stock solution of cyprodinil and CGA249287. Stock solutions were stored in refrigerator E-109.

3.4.3 Preparation of Fortification Standard Solutions

Mixed fortification standards were prepared by taking an approximately 1-mL (0.2-mL) aliquot of stock standard solutions and diluting them to 20-mL (25-mL). Fortifications used in this method validation are as follows:

Matrix	Stock Standard Concentration (ng/µL)	Aliquot Volume (mL)	Dilution Volume (mL)	Final Concentration (ng/µL)
Water	1	2	20	0.1
	0.1	2	20	0.01

3.4.4 Preparation of Calibration Standard Solutions

Mixed-standard calibration solutions were prepared by volumetrically diluting intermediate standard solutions with HPLC water. The following table is a summary of the actual preparation of calibration solutions:

Intermediate Concentration (ng/µL)	Aliquot Volume (mL)	Final Volume (mL)	Final Concentration (ng/µL)
1.0	0.5	50	0.01
0.01	4	20	0.002
0.01	2	20	0.001
0.01	1	20	0.0005
0.01	0.4	20	0.0002
0.01	0.5	50	0.0001
0.001	1	20	0.00005
0.001	0.4	20	0.00002

4.0 ANALYTICAL PROCEDURE

Each validation set included a reagent blank, two control water samples, five control water samples fortified at LOQ, and five control samples fortified at 10× LOQ.

4.1 Extraction

1. An 8-mL aliquot of the control water sample was measured into a 10-mL volumetric flask.
2. Untreated control water samples were fortified using 0.1 mL of the appropriate fortification standard at LOQ (0.1 ppb) and 10×LOQ (1.0 ppb) concentrations as per the method.
3. After fortification the sample was brought to 10-mL in a volumetric flask.
4. Sample was shaken vigorously for 10 seconds and vortexed to mix well.

4.2 Final Fraction

1. An aliquot (approximately 1.5 mL) was transferred from the sample to an autosampler vial.
2. Final determination as done by LC-MS/MS.

4.3 Instrumentation/Operating Conditions

4.3.1 Chromatographic Conditions

HPLC Instrument:	Agilent 1200 SL			
Column:	Synergi 4u Hydro-RP, 75 mm × 2 mm S/N 454942-2			
Column temperature:	40°C			
Injection volume:	10 µL			
Flow rate:	0.5 mL/min			
Mobile phase A:	Optima Water			
Mobile phase B:	Optima Methanol			
Gradient Step Table:	Step	Time (min)	A (%)	B (%)
	0	0	99	1
	1	0.5	99	1
	2	3.5	1	99
	3	4.5	1	99
	4	4.6	99	1
	5	5.5	99	1

4.3.2 Mass Spectrometer Conditions

Mass Spectrometer:	Agilent 6490 Series QQQ
Ion Mode:	ESI+Agilent Jet Stream
Polarity:	Positive
Gas Temp (°C):	150 °C
Gas Flow (L/min):	14
Nebulizer (psi):	45
Sheath Gas Heater:	300
Sheath Gas Flow:	12
Capillary (V):	3000
V Charging:	1500
Scan type:	MRM

MRM Conditions	Q1 *m/z*	Q3 *m/z*	Retention Time (min)	Dwell time	Frag (V)	CE (V)	Cell Acc (V)
Quantification Ions							
Cyprodinil	226.14	93.00	3.8	100	380	36	7
CGA249287	150.11	66.90	2.9	100	380	40	7
Confirmation Ions							
Cyprodinil	226.14	77.00	3.8	100	380	56	7
CGA249287	150.11	118.00	2.9	100	380	24	7

4.4 Data Acquisition

Peak integration and peak area count quantitation were performed by MassHunter (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 were calculated in LIMS and reported in Microsoft® Office Excel spreadsheets. Standard deviations and relative standard deviations were calculated and reported in Microsoft® Office Excel spreadsheets.

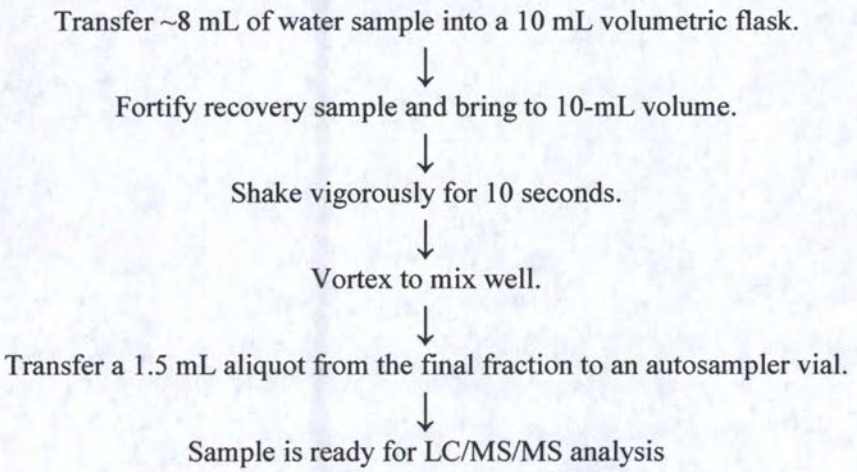
5.0

5.1 Method Establishment/Pre-Validation Evaluation

Initially, the mass spectrometer was optimized by infusing analyte standards to determine the optimum instrument operation parameters. Using these optimized instrument parameters, the retention times of the analytes, instrument detection limits and response linearity were established by injecting a series of calibration reference standards.

Prior to analysis of actual validation samples, a reagent blank and untreated control samples were analyzed to determine if interferences were present near the retention time of the analytes. The results of these evaluations indicated the selected control samples contained no detectable residues of cyprodinil and CGA249287 and had no peaks which might interfere with targeted analyte responses.

APPENDIX 1 Method Flow Chart



APPENDIX 5 Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system (MassHunter B.04.01). The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation for quantitation. LIMS was used to calculate the ppb and percent recovery and presented in Microsoft® Excel. The following equations were used for quantitation:

The following equations are used for residue calculations within MassHunter:

a) Calibration curve $y = mx + b$: Solving for x: $x = \frac{y - b}{m}$

Where, m = Slope
b = y-intercept
x = Amount found (ng)
y = Peak area

b) **Amount of sample injected (mL)** $= \frac{\text{Sample amt. (mL)} \times \text{Inj. size (mL)}}{\text{Final sample vol. (mL)}}$

c) **Analyte concentration (ppb)** $= \frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}}$

d) **Percent recovery** $= \left(\frac{\text{ppb in sample} - \text{ppb in control}}{\text{ppb added}}\right) \times 100$

As an example, calculations to obtain the percent recovery in control water sample from WO-14062511 fortified with cyprodinil in lab code 14062511-Recovery1-1. The calculations are shown below:

a) Calibration curve: $y = (12955764.820102) * x + 1523.712916$

Solving for x: $x = \frac{12056 - 1523.712916}{12955764.820102} = 0.000813$ ng

b) **Amount of sample injected (mL)** $= \frac{10.0\text{ mL} \times 0.01\text{ mL}}{10.0\text{ mL}} = 0.01$ mL

c) **Analyte concentration (ppb)** $= \frac{0.000813\text{ ng}}{0.01\text{ mL}} = 0.081294$ ppb

Average residue found in the untreated sample (lab code: 140625001-002B and 140625001-002C) = 0.000000 ppb

d) **Percent recovery** $= \left(\frac{0.081294 \text{ ppb} - 0.000000 \text{ ppb}}{0.1 \text{ ppb}}\right) \times 100 = 81.3\%$