2.0 INTRODUCTION

The purpose of this study was to conduct an independent laboratory validation of Syngenta Analytical Method GRM046.01A entitled "Thiabendazole – Residue Method for the Determination of Thiabendazole in Water," as written.

This study was designed to satisfy harmonized guideline requirements described in US EPA OCSPP 850.6100[1]. This study complied with the EC SANCO/3029/99 rev. 4 (2000)[2,] EC SANCO/825/00 rev. 8.1 (2010)[3], and OECD ENV/JM/MONO(2007)17[5] guidelines. This study was conducted in compliance with US EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160, which are compatible with the OECD Principles of Good Laboratory Practice (as revised in 2007).

The residue analytical method is suitable for the determination of Thiabendazole in water. Surface and ground water were selected for evaluation in this validation study to meet all requirements.

The method was successfully validated for Thiabendazole at 0.05 µg/L (LOQ) and 0.50 µg/L (10X LOQ) in ground water (drinking water) and surface water using external solvent calibration.

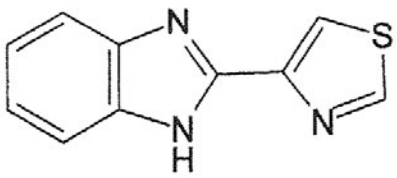
To summarize the method, 50 mL of water samples are acidified with HCl. Samples are processed through an Oasis HLB SPE column and eluted with MeOH. Eluate is evaporated to dryness and re-dissolved in ACN/10 mM NH_4OAc 60/40 v/v. The sample set was submitted for LC-MS/MS analysis. The limit of quantitation (LOQ) is 0.05 µg/L (ppb) for Thiabendazole.

The analytical procedure was performed as written with the following exceptions. The HPLC column listed in the method was no longer available for purchase and an HPLC column with similar length and column chemistry was used. Flow rate of the method was increased from 1 mL/min to 1.5 mL/min to adapt the method to the alternative column used. The ground water used was free of particulate matter and column reservoirs without frits were used. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110m filter paper after collection and column reservoirs without frits were used.

3.0 MATERIALS AND METHODS

3.1 Test Substance/Reference Substance

The test substance, Thiabendazole, was received on July 18, 2016 from Syngenta Crop Protection, Greensboro, North Carolina. The following information was provided:

Compound Structure	
Syngenta Code:	MK360
Common Name:	Thiabendazole
CAS Name:	1H-benzimidazole, 2-(4-thiazolyl)-
CAS Number:	148-79-8
IUPAC Name:	2-thiazol-4-yl-1H-benzimidazole
Batch Number:	WRS 1328/1
Molecular Weight:	201.3
Structural Formula	$C_{10}H_7N_3S$
Storage Conditions:	< 30°C
Purity:	98.8%
Recertification Date:	End of March 2017

Upon receipt, the test substance (Thiabendazole) was stored at room temperature in the original container. Concentrations were adjusted for the purity of the test substance.

All solutions made from the test substance (analytical standard) were stored according to the method.

3.2 Test Systems

The test systems evaluated in this study were surface water and ground (drinking) water. These matrices were chosen because they are representative of the water the method is designed for.

Approximately 6L of Surface water was collected from Musconetcong River at Waterloo Road at Saxton Falls in New Jersey, United States on August 17, 2016. Upon arrival at Symbiotic Research on the same date, surface water was stored refrigerated. The following day, the surface water was removed from and returned to refrigerator following filtration with Whatman Qualitative Circles, Grade 1.

Groundwater (bottled natural spring water) was purchased from a local grocer in Chester, NJ on August 18, 2016, and stored room temperature in the laboratory following arrival at Symbiotic Research.

Refrigerator storage temperatures were monitored on a daily basis and were typically at *c.a.* 4.0°C. Surface water was stored refrigerated, except for the periods during which the matrix was aliquoted for analysis. Groundwater was stored at room temperature for the entire duration of the study.

3.3 Equipment and Reagents/Supplies

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

3.3.1 Equipment

Balance:	Mettler Toledo Microbalance, Model AT20
HPLC:	Hewlett Packard Series 1100 Modular HPLC System Agilent Technology, Wilmington, DE. Equipped with Degasser, Binary Pump, Autosampler, Column Oven, DAD.
HPLC column:	Agilent Zorbax 300-SCX, 4.6 x 150mm, 5-micron

3.3.2 Reagents

Acetonitrile:	HPLC Grade (GC Chemical)
Methanol:	HPLC Grade (EMD Millipore)
Hydrochloric Acid:	ACS Grade (EMD)
Formic Acid:	ACS Grade (EMD)
Ammonium Acetate:	BioUltra ≥ 99.0% (Sigma Aldrich)
Water (H_2O):	LC-MS Grade (Fluka via Sigma Aldrich)

3.4 Preparation of Standard Solutions

The preparation of Thiabendazole standard solutions used for this study is described below. The solutions were stored as recommended in the method when not in use (frozen, *c.a.* 20°C).

3.4.1 Stock Standard Solution

A small amount of Thiabendazole reference substance was added to a pre-tared small glass vial and the weight (4.994 milligrams) was recorded. The volume of methanol (24.670 mL) needed to make a stock standard solution of Thiabendazole having a concentration of 200 µg/mL was calculated using the equation in section 2.3.1 of the method GRM046.01A. Two (2) mL of methanol was added to the vial and contents mixed by vortex and transferred to an appropriate sized larger vial. This step was repeated a total of three (3) times. The remaining volume of methanol was added to the larger vial and contents mixed by vortex to give a final concentration of 200 µg/mL.

3.4.2 Intermediate and Fortification Standard Solutions

Fortification Standard Solutions

10-µg/mL:	0.5 mL of a 200-µg/mL Thiabendazole stock standard solution was transferred to a 10-mL volumetric flask. The contents were brought to volume with methanol and mixed well.
1.0-µg/mL:	1.0 mL of a 10-µg/mL Thiabendazole stock standard solution was transferred to a 10-mL volumetric flask. The contents were brought to volume with methanol and mixed well.
0.1-µg/mL:	1.0 mL of a 1.0-µg/mL fortification solution was transferred to a 10-mL volumetric flask. The contents were brought to volume with methanol and mixed well.
0.01-µg/mL:	1.0 mL of a 0.1-µg/mL fortification solution was transferred to a 10-mL volumetric flask. The contents were brought to volume with methanol and mixed well.

3.4.3 HPLC (Calibration) Standard Solutions

Calibration standards were prepared from the fortification solutions and were stored frozen when not in use.

20 µg/L:	0.2 mL of a 1.0 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).
15 µg/L:	0.15 mL of a 1.0 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).
5 µg/L:	0.05 mL of a 1.0 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).

2.5 µg/L:	0.25 mL of a 0.1 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).
1.25 µg/L:	0.125 mL of a 0.1 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).
0.5 µg/L:	0.05 mL of a 0.1 µg/mL fortification solution diluted to 10 mL with ACN/10 mM NH4OAc (60/40, v/v).

3.5 Analytical Method

Analytical method GRM046.01A was successfully independently validated in this study. See Appendix 1 for the complete text of the method. The following is a summary of that method:

> 50 mL of water samples are acidified with HCl. Samples are processed through an Oasis HLB SPE column and eluted with MeOH. Eluate is evaporated to dryness and re-dissolved in 2 mL ACN/10 mM NH_4OAc 60/40 v/v. An aliquot of this sample was transferred to an autosampler vial. The LC vial was capped and submitted for LC-MS/MS analysis. The limit of quantitation (LOQ) is 0.05 µg/L (ppb) for Thiabendazole.

The analytical procedure was performed as written with the following exceptions. The HPLC column listed in the method was no longer available for purchase and an HPLC column with similar length and column chemistry was used. Flow rate of the method was increased from 1 mL/min to 1.5 mL/min to adapt the method to the alternative column used. The groundwater used was free of particulate matter and column reservoirs without frits were used. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110m filter paper after collection and column reservoirs without frits were used.

Residue calculations were performed as specified in the analytical method and were conducted using Analyst (version 1.4.2) software to prepare the calibration curve with 1/x weighting. The calculation worksheet can be found in Appendix 3.

3.5.1 Fortifications

Untreated surface and ground water samples were fortified using microliter amounts of the appropriate fortification standard for LOQ and 10X LOQ concentrations as per method. Fortifications used in this method validation are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/mL)	Final Volume (mL)	Final Conc. (µg/L)	Replicates
Surface Water	250	0.01	50	0.05 (LOQ)	5
Groundwater	250	0.01	50	0.05 (LOQ)	5
Surface Water	250	0.1	50	0.5 (10X LOQ)	5
Groundwater	250	0.1	50	0.5 (10X LOQ)	5

After fortification, the sample was mixed thoroughly before acidification.

3.6 Instrumentation Conditions

All samples were analyzed by LC-MS/MS detection. Typical conditions were as follows:

Chromatography Conditions

HPLC System	:	Hewlett Packard Series 1100 Modular HPLC System. Equipped with Degasser, Pump, Autosampler, Column Oven, DAD.
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software
Column	:	Agilent Zorbax 300-SCX, 4.6 x 150mm, 5-micron
Column Oven Temperature	:	40°C
Injection volume	:	10 µL
Stop Time	:	9.1 min
Injection protocol	:	Analyze calibration standard after 3 to 4 sample Injections
Mobile phase	:	Isocratic acetonitrile/10 mM NH4OAc 60/40 v/v

Mobile Phase Composition

Time (min)	%A	%B	Flow Rate (mL/min)
0.0	100	0	1.5
9.1	100	0	1.5

Mass Spectrometer Conditions

Ion Source Parameters:

Ionization Mode	Positive (+)
Curtain Gas (CUR)	12
Collision Gas (CAD)	7
IonSpray Voltage (V)	3000
Temperature (TEM)	600
Ion Source Gas 1 (GS1)	55
Ion Source Gas 2 (GS2)	50
Declustering Potential (DP)	66
Entrance Potential (EP)	10

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

MRM Operating Parameters:

Thiabendazole	MS/MS Transition	Time (msec.)	CE (Volts)	CXP (Volts)
Quantification	202 → 175	300	37	32
Confirmation	202 → 131	300	47	24

3.7 Modifications, Interpretations, and Critical Steps

The analytical procedure was performed as written with the following exceptions. The HPLC column listed in the method was no longer available for purchase and an HPLC column with similar length and column chemistry was used. Flow rate of the method was increased from 1 mL/min to 1.5 mL/min to adapt the method to the alternative column used. The groundwater used was free of particulate matter and column reservoirs without frits were used. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110m filter paper after collection and column reservoirs without frits were used.

3.8 Statistics

Statistical methods used were limited to calculations of the mean, range, standard deviation, 1/x weighting of linear regression and relative standard deviation. Software programs, Microsoft Excel® and Analyst (version 1.4.2), were employed to develop all regression analysis and statistical data.

4.0 RESULTS

4.1 Pre-Validation Evaluations

Prior to analysis of actual validation samples, the control samples initially selected for use in the study were analyzed per the method to determine if any interferences were present in the area of Thiabendazole. The result of this evaluation indicated that the control samples were free of any interference that would affect the analyte responses.

Control Suitability Evaluation		
MS/MS Transition	202 → 175	202 → 131
Matrix	**Residue (µg/L)[a]**	
Surface Water	0.00234	0.00389
Ground Water	ND	

[a]ND = none detected, no observable chromatographic response

4.4 Potential Interferences

Interferences were not observed in relation to LOQ (limit of quantification) in the matrices tested in this validation.

No significant matrix suppression or enhancement in the water matrices tested was observed.

4.5 Time Requirements

A single analyst can complete one (1) sample set consisting of thirteen (13) samples in one (1) working day (8 hours).

4.6 Protocol/SOP/Method Amendments and/or Deviations

Protocol was amended on August 19, 2016 to correct a change in sponsor address and an incorrect study number.

5.0 CIRCUMSTANCES AFFECTING DATA

No circumstances occurred during this validation that affected the quality or integrity of the data.

6.0 CONCLUSION

Syngenta Analytical Method GRM046.01A entitled "Thiabendazole – Residue Method for the Determination of Thiabendazole in Water," was successfully independently validated on the 1st attempt using the analytical procedures as written.

The method was demonstrated to be suitable for the determination of Thiabendazole in water at an LOQ of 0.05 µg/L (ppb) and 10X LOQ 0.50 µg/L (ppb).

TABLE 1 Characterization Data

Water Type	Source	pH	Calcium (ppm)	Magnesium (ppm)	Total Hardness as CaCO3 (mg/L)	Silt Content (ppm)	Dissolved Organic Carbon (ppm)
Surface Water	Musconetcong River, Saxton Falls	7.9	33	13	138	10	4.7
Groundwater	Evian bottled mineral water	7.8	84	27	323	6	0.6