1.0 INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of etridiazole and two major metabolites (3-carboxylic acid-5-ethoxy-1,2,4-thiadiazole (3-Carb-T) and 3-dichloromethyl-5-ethoxy-1,2,4-thiadiazole (3-DCMT)) in two different soil types, silt loam soil and clay loam soil. The analytical method was validated with regards to specificity, linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), and method detection limit (MDL).

The method was validated by fortification of silt loam soil and clay loam soil with etridiazole, 3-Carb-T, and 3-DCMT at concentrations of 50.0 and 500 µg/kg. Etridiazole and 3-DCMT recovery samples were extracted with 75:25 dichloromethane:acetone (v:v) followed by dilution into the calibration standard range with internal standard diluent. All samples were analyzed by automated injection using gas chromatography equipped with mass spectrometry detection (GC-MSD). 3-Carb-T recovery samples were extracted with 20:80 acetonitrile:purified reagent water (v:v) then further processed by anion exchange solid phase extraction (SPE), and eluted with 2% trifluoroacetic acid in methanol. Samples were diluted into the calibration standard range with 20:80:0.1 aectonitrile:purified reagent water:trifluoroacetic acid (v:v:v). All 3-Carb-T recovery samples were analyzed by automated injection using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

The study was initiated on 4 August 2017, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental portion of the validation was conducted from 14 September 2017 to 3 November 2017 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All original raw data, the protocol, and the final report produced during this study are stored in Smithers Viscient's archives at the above location.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this validation study followed those described in the Smithers Viscient protocol entitled "Validation of the Analytical Method for the Determination of Etridiazole and its Metabolites in Soil Matrices by LC-MS/MS and GCMS" (Appendix 1). The study was conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40 CFR Part 160 (U.S. EPA, 1989) and the OECD principles on GLP (OECD, 1998), and followed the guidance documents SANCO/825/00 REV 8.1 (EC, 2010) and OCSPP 850.6100 (U.S. EPA, 2012).

2.2 Test Substances and Internal Standard

2.2.1 Test substances

The test substance, etridiazole, was received on 13 June 2016 from Arysta LifeScience, Canada, Inc., Ontario, Canada. The following information was provided:

Name:	etridiazole
Synonyms:	5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole; etridiazole technical
Lot No.:	2758-31-RRG
CAS No.:	2593-15-9
Purity:	99.5 (± 0.10)% (Certificate of Analysis, Appendix 2)
Expiration Date:	30 November 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8327) was stored in a refrigerator in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, etridiazole acid, was received on 13 June 2016 from Arysta LifeScience, Canada, Inc., Ontario, Canada. The following information was provided:

Name:	etridiazole acid
Synonyms:	1,2,4-thiadiazole-3-carboxylic acid, 5-ethoxy-; 3-Carb-T
Lot No.:	2840-89-RRG
CAS No.:	67472-43-9
Purity:	99.9% (w/w) (Certificates of Analysis, Appendix 2)
Expiration Date:	28 February 2020

Upon receipt at Smithers Viscient, the test substance (SMV No. 8328) was stored in a freezer in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, DCE, was received on 13 June 2016 from Arysta LifeScience, Canada, Inc., Ontario, Canada. The following information was provided:

Name:	DCE
Synonyms:	1,2,4-thiadiazole, 3-(dichloromethyl)-5-ethoxy-; T-03; 3-DCMT
Lot No.:	2840-77-RRG
CAS No.:	Not Listed
Purity:	99.3% (Certificate of Analysis, Appendix 2)
Expiration Date:	27 February 2020]

Upon receipt at Smithers Viscient, the test substance (SMV No. 8329) was stored in a refrigerator in the original container. Concentrations were adjusted for the purity of the test substance.

Determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substances, and archival of a sample of the test substances are the responsibility of the Study Sponsor.

2.2.2 Internal Standard

The internal standard, benzophenone, was received on 31 March 2017 from Sigma Aldrich, Allentown, Pennsylvania. The following information was provided:

Name:	benzophenone
Synonym:	diphenyl ketone
Batch No.:	MKBT8749V
CAS No.:	119-61-9
Purity:	100% (Certificate of Analysis, Appendix 2)
Quality Release Date:	10 February 2015

Upon receipt at Smithers Viscient, the internal standard (SMV No. 8844) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the internal standard.

2.3 Reagents

1. Acetonitrile:	EMD, reagent grade
2. Dichloromethane:	EMD, reagent grade
3. Acetone:	EMD, reagent grade
4. Methanol:	EMD, reagent grade
5. Trifluoracetic Acid:	Sigma, reagent grade
6. Ammonium Hydroxide:	J.T Baker, reagent grade
7. Benzophenone:	Sigma, reagent grade
8. Purified reagent water:	Prepared from a Millipore Milli-Q Direct 8 water purification system (meets ASTM Type II requirements)

Reagents of similar grade and comparable purity may be substituted for the specific reagents above in future testing with this method as long as acceptable performance is demonstrated.

2.4 Equipment

1. Instrument (LC-MS/MS):	MDS Sciex API 5000 mass spectrometer equipped with an ESI Turbo V source Shimadzu SIL-20ACRX autoinjector Shimadzu DGU-20A5R vacuum degasser

	Shimadzu LC-20ADXR solvent delivery pumps Shimadzu CTO-20AC column compartment Shimadzu CBM-20A communications bus Analyst 1.6 software for data acquisition
2. Instrument (GC-MSD)	Agilent 6890 series gas chromatograph Agilent 7683 series autosampler Agilent 7683 series injector Agilent 5973 series mass selective detector (MSD)
3. Balances:	Mettler PJ-3000, Mettler Toledo XS205
4. Centrifuges:	Beckman Allegra X-12, Eppendorf 5417C
5. Shaker table:	VWR 3500STD
6. Moisture balance:	Mettler Toledo HB43-S
7. Laboratory equipment:	volumetric flasks, disposable glass pipets, positive displacement pipets, graduated cylinders, stir bars, stir plates, vortexers, autosampler vials, 50-mL centrifuge tubes, amber Wheaton bottles, 45-mL glass vials with PTFE caps, Waters MAX SPE columns, and amber glass bottles with Teflon-lined caps

Other equipment or instrumentation may be used but may require optimization to achieve the desired separation and sensitivity.

2.5 Test Soil

The matrices used during this method validation were silt loam soil and clay loam soil.

The soils used for the method validation were silt loam soil (SMV Lot No. 13SEP17SOIL-B) from Jackson, Iowa, and clay loam soil (SMV Lot No. 12DEC16SOIL-B) from Grand Forks, North Dakota. Prior to testing, soil moisture content was determined to be 13.37% for the silt loam soil and 18.84% for the clay loam soil using a Mettler Toledo HB43-S moisture analyzer. Soil characterization data are listed in the table below.

Soil Type	% Sand, Silt, Clay	Bulk Density (gm/cc)	CEC (meq/100 g)	% Organic Matter (Walkley Black)	pH in 1:1 soil:water Ratio
Silt Loam	24, 60, 16	0.92	12.1	3.9	7.2
Clay Loam	40, 28, 32	0.99	19.2	5.6	5.4

Soil Characterized by Agvise Laboratories, Northwood, North Dakota.

2.6 Preparation of Liquid Reagent and Mobile Phase Solutions

All volumes can be scaled up or down as necessary; however, the proportions must remain the same.

A 75:25 dichloromethane:acetone (v:v) liquid reagent solution was typically prepared by combining 250 mL of acetone, and 750 mL of dichloromethane. The solution was mixed using a stir bar and stir plate for five minutes.

A 0.1% trifluoroacetic acid in purified reagent water mobile phase solution was typically prepared by adding 1.00 mL of trifluoroacetic acid to 1000 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes, then degassed under vacuum with sonication for ten minutes.

A 0.1% trifluoroacetic acid in acetonitrile mobile phase solution was typically prepared by adding 1.00 mL of trifluoroacetic acid to 1000 mL of acetonitrile. The solution was mixed well using a stir bar and stir plate for five minutes, then degassed under vacuum with sonication for ten minutes.

A 30:30:40 acetonitrile:methanol:purified reagent water (v:v:v) autosampler needle wash solution was typically prepared by combining 1200 mL of acetonitrile, 1200 mL of methanol, and 1600 mL of purified reagent water.

A 20:80 acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 200 mL of acetonitrile, and 800 mL of purified reagent water. The solution was mixed using a stir bar and stir plate for five minutes.

A 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v) liquid reagent solution was typically prepared by combining 100 mL of acetonitrile, 400 mL of purified reagent water, and 0.500 mL of trifluoroacetic acid. The solution was mixed using a stir bar and stir plate for five minutes.

A 2% trifluoroacetic acid in methanol liquid reagent solution was typically prepared by combining 50.0 mL of methanol, and 1.00 mL of trifluoroacetic acid. The solution was vortex mixed for thirty seconds.

2.7 Preparation of Stock Solutions

All volumes and masses can be scaled up or down as necessary; however, the proportions must remain the same.

Primary stock solutions were typically prepared as described in the table below.

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
8327O	0.02519	0.02506	Acetone	25.0	1000	Sub-stock solution
8328M	0.02508	0.02505	Acetonitrile	25.0	1000	Secondary stock solutions
8328N	0.02510	0.02507	Acetonitrile	25.0	1000	Secondary stock solutions
8329I	0.02527	0.02509	Acetone	25.0	1000	Sub-stock solution
Internal Standard						
8844-1F	0.0500	0.0500	Acetone	50.0	1000	Secondary stock solutions

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
8328M	1000	0.500	50.0	Acetonitrile	8327M-1	10.0	Sub-stock solution and 10X LOQ recovery samples
8328N	1000	0.500	50.0		8328N-1	10.0	Sub-stock solution
Internal Standard							
8844-1F	1000	0.500	50.0	Acetone	8844-1F-1	10.0	Internal standard diluent

Sub-stock solutions were typically prepared as described in the table below.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
8328M-1	10.0	1.00	10.0	Acetonitrile	Tech Stk 1	1.00	LOQ and 10X LOQ recovery samples
8328N-1	10.0	1.00	10.0	Acetonitrile	Ana Stk 1	1.00	Calibration Standards
8327O	1,000	0.100	10.0	Acetone	Mixed Stock 1	10.0	Sub-stock solution and LOQ recovery samples
8329I	1,000	0.100					
Mix-Stk 1	10.0	1.00	10.0	Acetone	Mixed Stock 2	1.00	calibration standards

An internal standard diluent solution was typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Diluent Solvent	ID	Internal Standard Concentration (µg/L)
Internal Standard						
8844-1F-1	10.0	0.100	500	Acetone	IS Dil	2.00

All primary and secondary stock solutions were stored refrigerated (2 to 8˚C) in amber glass bottles fitted with Teflon-lined caps. Sub-stock solutions were prepared fresh on the day of use and discarded after use.

2.8 Preparation of Calibration Standards

2.8.1 Calibration Standards – Etridiazole and 3-DCMT

Calibration standards were prepared in internal standard diluent by fortifying with the 1.00 mg/L test substance mixed sub-stock solution to yield test substance concentrations of 0.750, 1.00, 1.50, 2.00, 5.00, and 7.50 µg/L. This procedure is detailed in the table below.

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Standard Concentration (µg/L)	Sample ID
Mixed Stock 2	1.00	0.0150	20.0	0.750	Std 1
		0.0200	20.0	1.00	Std 2
		0.0150	10.0	1.50	Std 3
		0.0200	10.0	2.00	Std 4
		0.0500	10.0	5.00	Std 5
		0.0750	10.0	7.50	Std 6

2.8.2 Calibration Standards – 3-Carb-T

Calibration standards were prepared in 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v) by fortifying with the 1.00 mg/L test substance sub-stock solution to yield test substance concentrations of 1.00, 2.00, 3.00, 5.00, 7.50, and 10.0 µg/L. This procedure is detailed in the table below.

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Standard Concentration (µg/L)	Sample ID
Ana Stk 1	1.00	0.0100	10.0	1.00	Std 1
		0.0200	10.0	2.00	Std 2
		0.0300	10.0	3.00	Std 3
		0.0500	10.0	5.00	Std 4
		0.0750	10.0	7.50	Std 5
		0.100	10.0	10.0	Std 6

2.8.3 Calibration Standards – Matrix Effects Etridiazole and 3-DCMT

Calibration standards used to assess possible matrix effects were prepared as follows by fortifying with the 1.00 mg/L test substance mixed sub-stock solution to yield test substance concentrations of 2.50 µg/L. These standards were quantified using matrix matched standards.

2.8.3.1 Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Mixed stock 2	1.00	0.0250	10.0	2.50	MM-Std 1-3
		0.0250	10.0	2.50	MM-Std 2-3
		0.0250	10.0	2.50	MM-Std 3-3

[a] Samples were diluted with the prepared matrix matched control (see Section 2.10 for extract preparation and dilution procedures).

2.8.3.2 Non Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Mixed stock 2	1.00	0.0250	10.0	2.50	Std A-3
		0.0250	10.0	2.50	Std B-3
		0.0250	10.0	2.50	Std C-3

[a] Samples were prepared in IS Dil.

2.8.4 Calibration Standards – Matrix Effects 3-Carb-T

Calibration standards used to assess possible matrix effects were prepared as follows by fortifying with the 1.00 mg/L test substance mixed sub-stock solution to yield test substance concentrations of 5.00 µg/L. These standards were quantified using matrix matched standards.

2.8.4.1 Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Ana Stk 1	1.00	0.0250	5.00	5.00	MM-Std 1
		0.0250	5.00	5.00	MM-Std 2
		0.0250	5.00	5.00	MM-Std 3

[a] Samples were diluted with the prepared matrix matched controls (see Section 2.10 for extract preparation and dilution procedures).

2.8.4.2 Non Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Ana Stk 1	1.00	0.0250	5.00	5.00	Std A
		0.0250	5.00	5.00	Std B
		0.0250	5.00	5.00	Std C

[a] Samples were diluted with 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v)

2.9 Sample Fortification and Preparation

2.9.1 Etridiazole and 3-DCMT

For each soil type, a total of 12 recovery samples (5.00 g dry weight) were weighed into individual 45-mL disposable glass vials with Teflon-lined caps and were fortified with the appropriate test substance mixed sub-stock solution at concentrations of 50.0 and 500 µg/kg. Five replicates were prepared for each concentration level. In addition, two samples were left unfortified to serve as controls and were extracted in the same fashion as the LOQ-level recovery samples. One matrix matched control sample was also prepared in order to provide a diluent to assess matrix effects. One reagent blank was also prepared (no test material or matrix) in order to assess interference from extraction solvents. The fortification procedure is detailed in the following tables.

Clay loam soil:

Sample ID 14088-6158-	Sample Type	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Sample Concentration (µg/kg)
67	Reagent Blank	NA[a]	NA	NA	0.00
68, & 69	Control	NA	NA	5.00	0.00
71, 72, 73, 74, & 75	LOQ	10.0	0.0250	5.00	50.0
76, 77, 78, 79, & 80	High	10.0	0.250	5.00	500

[a] NA = Not Applicable

Silt loam soil:

Sample ID 14088-6158-	Sample Type	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Sample Concentration (µg/kg)
95	Reagent Blank	NA[a]	NA	NA	0.00
96, & 97	Control	NA	NA	5.00	0.00
99, 100, 101, 102, & 103	LOQ	10.0	0.0250	5.00	50.0
104, 105, 106, 107, & 108	High	10.0	0.250	5.00	500

[a] NA = Not Applicable

2.9.2 3-Carb-T

For each soil type, a total of 12 recovery samples (5.00 g dry weight) were weighed into individual 50-mL Nalgene centrifuge tubes and were fortified with the appropriate test substance sub-stock and stock solutions at concentrations of 50.0 and 500 µg/kg. Five replicates were prepared for each concentration level. In addition, two samples were left unfortified to serve as controls and were extracted in the same fashion as the LOQ-level recovery samples. One reagent blank was also prepared (no test material or matrix) in order to assess interference from extraction solvents. The dosing procedure is detailed in the following tables.

Clay loam soil:

Sample ID 14088-6158-	Sample Type	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Sample Concentration (µg/kg)
122	Reagent Blank	NA[a]	NA	NA	0.00
123, & 124	Control	NA	NA	5.00	0.00
125, 126, 127, 128, & 129	LOQ	1.00	0.250	5.00	50.0
130, 131, 132, 133, & 134	High	10.0	0.250	5.00	500

[a] NA = Not Applicable

Silt loam soil:

Sample ID 14088-6158-	Sample Type	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Sample Concentration (µg/kg)
109	Reagent Blank	NA[a]	NA	NA	0.00
110, & 111	Control	NA	NA	5.00	0.00
112, 113, 114, 115, & 116	LOQ	1.00	0.250	5.00	50.0
117, 118, 119, 120, & 121	High	10.0	0.250	5.00	500

[a] NA = Not Applicable

2.10 Sample Extraction

2.10.1 Etridiazole and 3-DCMT

The samples were extracted with 30.0 mL aliquots of 75:25 dichloromethane:acetone (v:v) using 45-mL glass vials with PTFE lined caps, and they were placed on a shaker table for 30 minutes at 150 rpm. At the end of 30 minutes, the samples were centrifuged for 15 minutes at 1,200 rpm. A portion of each extract was concentrated under a gentle stream of nitrogen to incipient dryness (approximately 100 µL) prior to being reconstituted with internal standard diluent.

Clay loam soil:

Sample ID 14088-6158-	Sample Type	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
67	Reagent Blank	0.00	NA[c]	30.0	3.00	10.0	20.0
68, & 69	Control	0.00	5.00	30.0	3.00	10.0	20.0
70	MM Control	0.00	5.00	30.0	12.0	40.0	20.0
71, 72, 73, 74, & 75	LOQ	50.0	5.00	30.0	3.00	10.0	20.0
76, 77, 78, 79, & 80	High	500	5.00	30.0	0.300	10.0	200

[a] Extraction Solvent: 75:25 DCM:Acetone (v:v)
[b] Reconstitution Solvent: IS Dil
[c] NA = Not Applicable

Silt loam soil:

Sample ID 14088-6158-	Sample Type	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
95	Reagent Blank	0.00	NA[c]	30.0	3.00	10.0	20.0
96, & 97	Control	0.00	5.00	30.0	3.00	10.0	20.0
98	MM Control	0.00	5.00	30.0	12.0	40.0	20.0
99, 100, 101, 102, & 103	LOQ	50.0	5.00	30.0	3.00	10.0	20.0
104, 105, 106, 107, & 108	High	500	5.00	30.0	0.300	10.0	200

[a] Extraction Solvent: 75:25 DCM:Acetone (v:v)
[b] Reconstitution Solvent: IS Dil
[c] NA = Not Applicable

2.10.2 3-Carb-T

A 20.0-mL aliquot of 20:80 acetonitrile:purified reagent water (v:v) was added to each soil recovery sample (5.00 g dry weight) and samples were placed on a shaker table for 30 minutes at 150 rpm. The samples were then centrifuged at 3000 rpm for 10 minutes and the extracts were transferred to 50.0-mL volumetric flasks. The extraction and centrifugation procedures were repeated with an additional 20.0-mL aliquot of 20:80 acetonitrile:purified reagent water (v:v). The extracts were combined, taken to volume (50.0 mL) with 20:80 acetonitrile:purified reagent water (v:v) and mixed well. A 5.00-mL aliquot of each recovery sample extract was removed to be further processed by solid phase extraction.

Oasis Mixed-Mode Strong Anion Exchange (MAX) SPE columns (60 mg, 3 mL) were conditioned by rinsing with two column volumes of methanol followed by two column volumes of purified reagent water. The columns were not allowed to go dry until before elution. A 1.0-µL aliquot of ammonium hydroxide was added to each 5 mL sample. The samples were loaded onto the columns, and allowed to flow through under vacuum at no greater than 1 drop/sec. Each sample vessel and column was rinsed with 5.00-mL of purified reagent water and was loaded onto the column and allowed to flow through under vacuum at no greater than 1 drop/sec. Each sample vessel and column was rinsed with 5.00-mL of methanol and was loaded onto the column and allowed to flow through under vacuum at no greater than 1 drop/sec.

The water and methanol rinses were first used to rinse the recovery sample vessels and were then added to the SPE columns. The water eluates and rinsates were discarded. The columns were quickly dried under full vacuum. The test substance was eluted from the SPE columns with 3.00-mL of 2% trifluoroacetic acid in methanol under vacuum at no greater than 1 drop/sec and collected into glass conical vials. When eluting, the sorbent was saturated with elution solvent and allowed to sit for thirty seconds before applying vacuum. The samples were concentrated to incipient dryness under a gentle stream of nitrogen at 50.0˚C. The concentrated extracts were reconstituted in 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v) which was added to each sample with mixing and sonication (5 minutes) to aid in reconstitution. The sample processing is summarized in the table below.

Clay loam soil:

Sample ID 14088-6158-	**Sample Type**	**Nominal Concentration (µg/kg)**	**Dry Weight (g)**	**Final Volume[a] (mL)**	**Sample Volume (mL)**	**Final Volume[b] (mL)**	**Sample Volume (mL)**	**Final Volume[b] (mL)**	**Dilution Factor**
122	Reagent Blank	0.00	NA[c]	50.0	5.00	5.00	NA	NA	10.0
123, & 124	Control	0.00	5.00	50.0	5.00	5.00	NA	NA	10.0
MM-Std 1, 2, & 3	MM Control	0.00	NA	50.0	5.00	5.00	NA	NA	10.0
125, 126, 127, 128, & 129	LOQ	50.0	5.00	50.0	5.00	5.00	NA	NA	10.0
130, 131, 132, 133, & 134	High	500	5.00	50.0	5.00	5.00	1.00	10.0	100

[a] Extraction Solvent: 20:80 acetonitrile:purified reagent water (v:v)
[b] Reconstitution Solvent: 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v)
[c] NA = Not Applicable

Silt loam soil:

Sample ID 14088-6158-	Sample Type	Nominal Concentration (µg/kg)	Dry Weight (g)	Final Volume[a] (mL)	Sample Volume (mL)	Final Volume[b] (mL)	Sample Volume (mL)	Final Volume[b] (mL)	Dilution Factor
109	Reagent Blank	0.00	NA[c]	50.0	5.00	5.00	NA	NA	10.0
110, & 111	Control	0.00	5.00	50.0	5.00	5.00	NA	NA	10.0
MM-Std 1, 2, & 3	MM Control	0.00	NA	50.0	5.00	5.00	NA	NA	10.0
112, 113, 114, 115, & 116	LOQ	50.0	5.00	50.0	5.00	5.00	NA	NA	10.0
117, 118, 119, 120, & 121	High	500	5.00	50.0	5.00	5.00	1.00	10.0	100

[a] Extraction Solvent: 20:80 acetonitrile:purified reagent water (v:v)
[b] Reconstitution Solvent: 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v)
[c] NA = Not Applicable

2.11 Analysis

2.11.1 Instrument Conditions Etridiazole and 3-DCMT

The GC-MS/EI analysis was conducted utilizing the following instrumental conditions

GC Parameters:

Column: Agilent DB-5MS, 15 m × 0.250 mm × 0.25 µm
Temperature: 50 ºC (initial) and held for 2.00 minutes
Ramps:

Rate (ºC/min)	Final Temperature (ºC)	Hold Time (min)
45	250	0.00

Post Temperature: 300˚C
Post Time: 8.00
Run Time: 7.44 minutes
Injection Volume: 2.00 µL
Carrier Gas: Helium
Gas Flows: Constant flow of 1.0 mL/minute
Inlet Mode: Splitless, purge flow to 50.0 mL/minute at 1.00 minute
Inlet Temperature: 200 ºC
Retention Time: Etridiazole, approximately 6.1 minutes
3-DCMT, approximately 5.8 minutes

MSD Parameters:

Solvent Delay: 3.00 minutes

Selected Ion Monitoring:

Etridiazole:

Ion (m/z)	Dwell (msec)	Comments
211.00	50	Primary ion
185.00	50	Confirmation ion
183.00	50	Confirmation ion

3-DCMT:

Ion (m/z)	Dwell (msec)	Comments
143.00	50	Primary ion
184.00	50	Confirmation ion
186.00	50	Confirmation ion

Temperatures: MSD Transfer Line: 300 ºC
MS Quad: 150 ºC
MS Source: 230 ºC

2.11.2 Instrumental Conditions 3-Carb-T

The LC-MS/MS analysis was conducted using the following instrumental conditions:

LC Parameters:

Column:	Phenomenex Kinetex 5 µm EVO C18 50 x 2.1mm
Mobile Phase A:	0.1% trifluoroacetic acid in water
Mobile Phase B:	0.1% trifluoroacetic acid in acetonitrile

Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.500	98.0	2.0
0.50	0.500	98.0	2.0
2.00	0.500	0.0	100
3.00	0.500	0.0	100
3.10	0.500	98.0	2.0
4.00	0.500	98.0	2.0

Run Time:	4.0 minutes
Autosampler Wash:	30:30:40 acetonitrile:methanol:purified reagent water (v:v:v)
Column Temperature:	35 °C
Sample Temperature:	15 °C
Injection Volume:	50 µL
Retention Time:	approximately 1.5 minutes for 3-Carb-T

MS Parameters:

Instrument:	MDS Sciex API 5000 mass spectrometer
Ionization Mode:	Positive (+) ESI
Ion Spray Voltage:	5500 V
Scan Type:	MRM
Resolution Q1/Q3:	Unit/Unit
Source Temperature:	550 °C
Curtain Gas:	15.00
Ion Source – Gas 1/Gas 2:	70.00/70.00
Collision Gas:	4.00
Declustering Potential:	45.00

	Primary Transition	Confirmatory Transition
Q1/Q3 Masses (amu):	175.16/147.10	175.16/129.00
Dwell Time (milliseconds):	200	200
Entrance Potential:	4.00	10.00
Collision Energy:	15.00	23.00
Collision Cell Exit Potential:	15.0	15.0

Other instrumentation may be used but may require optimization to achieve the desired separation and sensitivity. It is important to note that the parameters above have been established for this particular instrumentation and may not be applicable for other similar equipment that may be used.

2.11.3 Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each recovery sample set: one set prior to analysis of the recovery samples, and the second set immediately following the analysis of the recovery samples. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

2.12 Evaluation of Precision, Accuracy, Specificity and Linearity

The accuracy was reported in terms of percent recovery of the LOQ and 10X LOQ recovery samples. Recoveries of 70.0 to 120% were considered acceptable. The precision was reported in terms of the standard deviation and relative standard deviation for the retention time, peak area based quantitation, and the percent recovery values of the LOQ and 10X LOQ recovery samples. RSD values less than or equal to 20% were considered acceptable for the recovery samples, while RSD values less than or equal to 2% were considered acceptable for the retention times. Specificity of the method was determined by examination of the control samples for peaks at the same retention time as etridiazole, 3-Carb-T, and 3-DCMT which might interfere with the quantitation of the analytes. A linear calibration curve was used for this testing. This calibration curve was evaluated based on the correlation coefficient (r^2) and the recoveries of the calibration standards.

2.13 Limit of Quantitation (LOQ)

The method was validated at the Limit of Quantitation (LOQ). This was defined as the lowest fortification level. Blank values (reagent blanks and untreated control samples) did not exceed 30% of the LOQ.

2.14 Limit of Detection (LOD) and Method Detection Limit (MDL)

The Limit of Detection (LOD) was calculated using three times the signal-to-noise value of the control samples. Representative calculations for the LOD can be found in Calculations.

The Method Detection Limit (MDL) was defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions. Representative calculations for the MDL can be found in Calculations.

3.0 Calculations

A calibration curve was constructed by plotting the analyte concentration (µg/L) of the calibration standards against the peak area of the analyte in the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of test substance in each recovery sample was calculated using the slope and intercept from the linear regression analysis, the detector response, and the dilution factor of the recovery sample. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

(1) $$y = mx + b$$

(2) $$DC\,(x) = \frac{(y - b)}{m}$$

(3) $$A = DC \times DF$$

where:

x	=	analyte concentration
y	=	detector response (peak area) from the chromatogram
b	=	y-intercept from the regression analysis
m	=	slope from the regression analysis
DC (x)	=	detected concentration (µg/kg) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample volume)
A	=	analytical result (µg/kg), concentration in the original sample

The LOD was calculated using the following equation:

$$(4) \qquad LOD = ((3xSN_{ctl})Resp_{LS}) \times Conc_{LS}$$

where:

SN_{ctl}	=	mean signal to noise in height of the control samples (or blanks)
$Resp_{LS}$	=	mean response in height of the two low calibration standards
$Conc_{LS}$	=	concentration of the low calibration standard
LOD	=	limit of detection for the analysis

The MDL was calculated using the following equation.

$$(5) \qquad MDL = MDL_{LCAL} \times DF_{CNTL}$$

where:

MDL_{LCAL}	=	the lowest concentration calibration standard
DF_{CNTL}	=	dilution factor of the control samples
MDL	=	minimum detection limit reported for the analysis of etridiazole, 3-Carb-T or 3-DCMT recovery samples