1.0   INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of ipconazole in soil and sediment. The method was validated (18 May to 16 June 2017) to quantify the concentrations of ipconazole present in recovery samples prepared in loamy sand soil and marine sediment. The analytical method was validated with regards to specificity, linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), method detection limit (MDL), and confirmation of analyte identification.

The method was validated by fortification of soil and sediment with ipconazole at concentrations of 50.0 (limit of quantitation, LOQ) and 500 µg/kg (10 × LOQ). All recovery samples were extracted with 90:10:0.1 acetonitrile:purified reagent water:formic acid (v:v:v). The soil and sediment recovery sample extracts were further diluted into the calibration standard range with 50:50 methanol:purified reagent water (v:v). All samples were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

The study was initiated on 9 May 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 on 18 May to 16 June 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 study followed those described in the Smithers Viscient protocol entitled "Validation of the Analytical Method for the Determination of Ipconazole in Soil and Sediment by LC-MS/MS" (Appendix 1). The study was conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40 CFR 160 (U.S. EPA, 1989) and the OECD principles

on GLP (OECD, 1998), and followed the guidance documents SANCO/825/00rev 8.1 (EC, 2010) and OCSPP 850.6100 (U.S. EPA, 2012).

2.2   Test and Reference Substances

2.2.1   Test Substance

The test substance, ipconazole technical, was received on 15 September 2016 from Kureha Corporation, Shinjuku-ku, Tokyo, Japan. The following information was provided:

Name:	ipconazole technical material
Lot No.:	89010
CAS No.:	125225-28-7
Purity:	96.7% w/w (as total ipconazole), 89.7% w/w (as ipconazole cc), and 7.0% w/w (as ipconazole ct) (Certificate of Analysis, Appendix 2)
Expiration Date:	24 November 2019

Upon receipt at Smithers Viscient, the test substance (SMV No. 8522) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance as total ipconazole.

2.2.2   Reference Substances

The reference substance, ipconazole cc isomer, was received on 15 September 2016 from Kureha Corporation, Shinjuku-ku, Tokyo, Japan. The following information was provided:

Name:	ipconazole cc
Lot No.:	G-00328
CAS No.:	115850-69-6
Purity:	99.5% (Certificate of Analysis, Appendix 2)
Expiration Date:	12 September 2021

Upon receipt at Smithers Viscient, the reference substance (SMV No. 8523) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the reference substance.

The reference substance, ipconazole ct, was received on 15 September 2016 from Kureha Corporation, Shinjuku-ku, Tokyo, Japan. The following information was provided:

Name:	ipconazole ct
Lot No.:	G-00329
CAS No.:	115937-89-8
Purity:	99.7% (Certificate of Analysis, Appendix 2)
Expiration Date:	9 September 2021

Upon receipt at Smithers Viscient, the reference substance (SMV No. 8524) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the reference substance.

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

2.3   Reagents

1. 0.1% Formic acid in purified
   reagent water: Fisher Chemical, reagent grade
2. 0.1% Formic acid in
   acetonitrile: Fisher Chemical, reagent grade
3. Formic acid: BDH, reagent grade
4. Methanol: EMD, reagent grade
5. Acetonitrile: EMD, reagent grade
6. Purified reagent water: Prepared from a Millipore MilliQ® Direct 8 water
                                 purification system (meets ASTM Type II requirements)

2.4   Instrumentation and Laboratory Equipment

1. Instruments: AB MDS Sciex API 4000 mass spectrometer equipped with
                                 an AB MDS Sciex ESI Turbo V source
                                 Shimadzu LC-20AD binary pumps
                                 Shimadzu DGU-20A3 vacuum degasser
                                 Shimadzu DGU-20A5R vacuum degasser

Shimadzu SIL-20ACHT autosampler
Shimadzu CTO-20AC column oven
Shimadzu CBM-20A communications bus
Analyst version 1.4.2 software for data acquisition

And

MDS Sciex API 4000 QTRAP® mass spectrometer
equipped with an ESI Turbo V source
Agilent 1200SL/G1379B vacuum degasser
Agilent 1200SL/G1312B binary pump
Leap HTS PAL autosampler
Agilent 1200SL/G1316B column thermostat
Analyst version 1.6.2 software for data acquisition

2.	Balances:	Mettler Toledo XS205; Mettler Toledo AG285; and Mettler Toledo PG-2002-S
3.	Centrifuges:	Beckman Allegra X-12; and Beckman 367160
4.	Moisture balance:	Sartorius Moisture Analyzer MA-45
5.	Shaker table:	Orbit Shaker Table 3520
6.	Laboratory equipment:	Positive displacement pipets, volumetric flasks, disposable glass vials, disposable glass pipets, centrifuge tubes, graduated cylinders, Pasteur pipets, vortexer, autosampler vials, and amber glass bottles with Teflon®-lined caps

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

2.5   Test Matrices

The soils used for the method validation were Rochester loamy sand soil (SMV Lot No. 012616A) from Rochester, Massachusetts and freshwater sediment (SMV Lot No. 040317) from Glen Charlie Pond, Wareham, Massachusetts. Prior to testing, soil moisture content was determined to be 20.37% for the Rochester loamy sand soil 56.20% for the freshwater sediment using a Sartorius moisture analyzer. Characterization of soil and sediment was performed by Agvise Laboratories, Northwood, North Dakota. 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
Loamy sand soil	78,18,4	1.06	9.7	4.9	6.8
Freshwater sediment	96, 4, 0	NA[a]	NA	2.0	5.2

[a]   NA = Not Applicable
Soil Characterized by Agvise Laboratories, Northwood, North Dakota.

2.6   Preparation of Liquid Reagent and Mobile Phase Solutions

The volumes listed in this section were those used during the validation. For future testing, the actual volumes used may be scaled up or down as necessary.

A 50:50 acetonitrile:purified reagent water (v:v) liquid reagent solution was prepared by combining 200 mL of acetonitrile and 200 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes.

A 90:10:0.1 acetonitrile:purified reagent water:formic acid (v:v:v) liquid reagent solution was prepared by combining 900 mL of acetonitrile, 100 mL of purified reagent water, and 1.00 mL of formic acid. The solution was mixed using a stir bar and stir plate for five minutes.

A 30:30:40 acetonitrile:methanol:purified reagent water (v:v:v) autosampler needle wash solution was prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water. The solution was mixed well before use.

A 10:90 acetonitrile: purified reagent water (v:v) autosampler needle wash solution was prepared by combining 500 mL of acetonitrile and 4500 mL of purified reagent water. The solution was mixed well before use.

2.7 Preparation of Stock Solutions

The volumes and masses listed in this section were those used during each separate validation. For future testing, the actual volumes and masses used may be scaled up or down as necessary.

Primary stock solutions were prepared as described in the table below:

Primary Stock ID	Amount of Substance Weighed (g), Net Weight	Amount of Substance Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration[a] (mg/L)	Primary Stock Use
Test Substance						
8522M	1.0341	1.0000	Methanol	50.0	20,000	Secondary stock solution
8522O	1.0362	1.0020		50.0	20,000	Secondary stock solution
Reference Substances						
8523F	0.0503	0.0500	Methanol	50.0	1000	Secondary stock solution
8524F	0.0502	0.0500		50.0	1000	Secondary stock solution
8523G	0.0251	0.0250		25.0	999	Secondary stock solution
8524G	0.0251	0.0250		25.0	1000	Secondary stock solution

[a] Concentration expressed as ipconazole cc/ct isomers.

Secondary stock solutions were prepared as per the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration[a] (mg/L)	Stock Use
Test Substance							
8522M	20,000	0.250	50.0	Methanol	8522M-1	100	Sub-stock solution
8522O	20,000	0.250	50.0		8522O-1	100	Sub-stock solution
Reference Substances							
8523F	1000	0.500	50.0	Methanol	8523F-1	10.0	Sub-stock solution
8524F	1000	0.500	50.0		8524F-1	10.0	Sub-stock solution
8523G	999	0.500	50.0		8523G-1	9.99	Sub-stock solution
8524G	1000	0.500	50.0		8524G-1	10.0	Sub-stock solution

[a] Concentration expressed as ipconazole cc/ct isomers.

Sub-stock solutions were prepared as per the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (μg/L)	Stock Use
Test Substance							
8522M-1	100	1.00	10.0		Tech Mix 1	9.28/0.72[a]	High-level recovery samples[b]
Tech Mix 1	9.28/0.72[a]	1.00	10.0	Methanol	Tech Mix 2	0.928/0.072[a]	LOQ-level recovery samples[b]
Tech Mix 2	0.928/0.072[a]	0.100	10.0		Tech Mix 3	0.00928/0.00072[a]	Matrix-effects standards[b]
8522O-1	100	1.00	10.0		Tech Mix 1	9.29/0.73[a]	High-level recovery samples[c]
Tech Mix 1	9.29/0.73[a]	1.00	10.0	Methanol	Tech Mix 2	0.929/0.073[a]	LOQ-level recovery samples[c]
Tech Mix 2	0.929/0.073[a]	0.100	10.0		Tech Mix 3	0.00929/0.00073[a]	Matrix-effects standards[c]
Reference Substances							
8523F-1	10.0	0.500	100	Methanol	Ana Mix 1	0.0500/0.00350[a]	Calibration standards
8524F-1	10.0	0.0350					
8523G-1	9.99	0.500	100	Methanol	Ana Mix 1	0.0500/0.00350[a]	Calibration standards
8524G-1	10.0	0.0350					

[a] Concentration expressed as ipconazole cc/ct isomers.
[b] Loamy sand soil samples
[c] Freshwater sediment samples

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 – Recovery Samples

Calibration standards were prepared in 50:50 methanol:purified reagent water (v:v) by fortifying with the 0.0500/0.00350 mg/L mixed ipconazole cc/ipconazole ct sub-stock solution to yield test substance concentrations listed in the table below.

Test Substance Stock ID	Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Standard Concentration (µg/L)	Sample ID
Ana Mix 1	0.0500/0.00350[a]	0.0200	20.0	0.0500/0.00350[a]	Std 1
		0.0200	10.0	0.100/0.00700[a]	Std 2
		0.0400	10.0	0.200/0.0140[a]	Std 3
		0.0600	10.0	0.300/0.0210[a]	Std 4
		0.0800	10.0	0.400/0.0280[a]	Std 5
		0.100	10.0	0.500/0.0350[a]	Std 6

[a]   Concentration expressed as ipconazole cc/ct isomers.

2.8.2   Matrix Effect Investigation

In an effort to observe any potential matrix effects, an aliquot of control sample final fraction was fortified in triplicate and analyzed at each transition. These matrix-matched standards were compared to non-matrix matched standards fortified at the same concentration. Calibration standards used to assess possible matrix effects were prepared as described in the following tables.

2.8.2.1   Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID[b]
Tech Mix 3	0.00928/0.00072[c]	0.100	10.0	0.0928/0.0072[c]	MM-Std A
		0.100	10.0	0.0928/0.0072[c]	MM-Std B
		0.100	10.0	0.0928/0.0072[c]	MM-Std C
Tech Mix 3	0.00929/0.00073[c]	0.100	10.0	0.0929/0.0073[c]	MM-Std D
		0.100	10.0	0.0929/0.0073[c]	MM-Std E
		0.100	10.0	0.0929/0.0073[c]	MM-Std F

[a]   Samples were diluted with the final fraction of the Control A for the loamy sand soil or Control C for the freshwater sediment following dilution in 50:50 methanol:purified reagent water (v:v); (see Section 2.9 for extract preparation and dilution procedures).
[b]   Sample ID codes included: A, B, and C (loamy sand soil); D, E, and F (freshwater sediment).
[c]   Concentration expressed as ipconazole cc/ct isomers.

2.8.2.2   Non-Matrix-Matched Standards

Test Substance Stock ID	Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Tech Mix 3	0.00928/0.00072[b]	0.100	10.0	0.0928/0.0072[b]	Sol-Std A
		0.100	10.0	0.0928/0.0072[b]	Sol-Std B
		0.100	10.0	0.0928/0.0072[b]	Sol-Std C
Tech Mix 3	0.00929/0.00073[b]	0.100	10.0	0.0929/0.0073[b]	Sol-Std D
		0.100	10.0	0.0929/0.0073[b]	Sol-Std E
		0.100	10.0	0.0929/0.0073[b]	Sol-Std F

[a]   Samples were diluted with 50:50 methanol:purified reagent water (v:v).
[b]   Concentration expressed as ipconazole cc/ct isomers.

2.9   Sample Fortification and Preparation

The recovery samples were prepared in two different matrices (loamy sand soil and freshwater sediment) with ipconazole technical material at concentrations of 50.0 and 500 µg/kg. For each soil type, a total of 12 recovery samples (5.00 g dry weight) were weighed into individual 50-mL centrifuge tubes 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 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.

Recovery samples in loamy sand soil:

Sample ID	Sub-Stock Concentration (mg/L)	Volume of Fortification (mL)	Dry Weight (g)	Fortified Concentration (µg/kg)
Reagent blk-1	NA[a]	NA	NA	0.00
Control A & B	NA	NA	5.00	0.00
LOQ A, B, C, D, & E	0.928/0.072[b]	0.250	5.00	46.4/3.6[b]
High A, B, C, D, & E	9.28/0.72[b]	0.250	5.00	464/36[b]

[a]   NA = Not Applicable
[b]   Concentration expressed as ipconazole cc/ct isomers.

Recovery samples in freshwater soil:

Sample ID	Sub-Stock Concentration (mg/L)	Volume of Fortification (mL)	Dry Weight (g)	Fortified Concentration (µg/kg)
Reagent blk-2	NA[a]	NA	NA	0.00
Control C & D	NA	NA	5.00	0.00
LOQ F, G, H, I, & J	0.929/0.073[b]	0.250	5.00	46.5/3.7[b]
High F, G, H, I, & J	9.29/0.73[b]	0.250	5.00	465/37[b]

[a] NA = Not Applicable
[b] Concentration expressed as ipconazole cc/ct isomers.

2.10   Soil Extraction

A 20.0-mL aliquot of 90:10:0.1 acetonitrile:purified reagent water:formic acid (v: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 90:10:0.1 acetonitrile:purified
reagent water:formic acid (v:v:v). The extracts were combined, taken to volume (50.0 mL) with
90:10:0.1 acetonitrile:purified reagent water:formic acid (v:v:v) and mixed well. The soil
recovery sample extracts were further diluted into the calibration standard range with
50:50 methanol:purified reagent water (v:v). Prior to analysis loamy sand samples were
centrifuged at 13,000 rpm for five minutes using low-binding centrifuge tubes. Sediment samples
were not centrifuged. All recovery samples were transferred to HPLC vials for analysis.
Secondary dilution volumes can be scaled up or down as necessary. The extraction and dilution
procedures are detailed below.

For loamy sand soil

Sample ID	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent blk-1	0.00	NA[c]	20.0	50.0	0.200	10.0	500
Control A	0.00	5.00	20.0	50.0	2.00	100[d]	500
Control B	0.00	5.00	20.0	50.0	0.200	10.0	500
LOQ A, B, C, D, & E	46.4/3.6[e]	5.00	20.0	50.0	0.200	10.0	500
High A, B, C, D, & E	464/36[e]	5.00	20.0	50.0	0.0750	10.0	1330

[a]   Extraction solvent: 90:10:0.1 acetonitrile:purified reagent water:formic acid (v:v:v)
[b]   Dilution solvent: 50:50 methanol:purified reagent water (v:v)
[c]   NA = Not Applicable
[d]   Increased volume for matrix investigation
[e]   Concentration expressed as ipconazole cc/ct isomers.

For freshwater sediment

Sample ID	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent blk-2	0.00	NA[c]	20.0	50.0	0.200	10.0	500
Control C	0.00	5.00	20.0	50.0	2.00	100[d]	500
Control D	0.00	5.00	20.0	50.0	0.200	10.0	500
LOQ F, G, H, I, & J	46.5/3.7[e]	5.00	20.0	50.0	0.200	10.0	500
High F, G, H, I, & J	465/37[e]	5.00	20.0	50.0	0.0750	10.0	1330

[a]   Extraction solvent: 90:10:0.1 acetonitrile:purified reagent water:formic acid (v:v:v)
[b]   Dilution solvent: 50:50 methanol:purified reagent water (v:v)
[c]   NA = Not Applicable
[d]   Increased volume for matrix investigation
[e]   Concentration expressed as ipconazole cc/ct isomers.

2.11   Analysis

2.11.1   Instrumental Conditions

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

LC parameters:

Column:	Waters XBridge C18, 3.5 µm, 2.1 × 100 mm
Mobile Phase A:	0.1% formic acid in reagent grade water
Mobile Phase B:	0.1% formic acid in acetonitrile
Gradient:	

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.400	50.0	50.0
4.00	0.400	50.0	50.0
7.00	0.400	30.0	70.0
7.10	0.400	0.00	100
9.00	0.400	0.00	100
9.10	0.400	50.0	50.0

Run Time:	10.5 minutes
Injector Rinse Solvent 1:	30:30:40 acetonitrile:methanol:reagent grade water (v:v:v)
Injector Rinse Solvent 2:	10:90 acetonitrile:purified reagent water (v:v) (freshwater sediment)
Column Temperature:	40 °C
Sample Temperature:	5 °C
Injection Volume:	25.0 µL
Retention Times:	see table below

		Retention Time	
Analyte	**Analysis**	**Loamy Sand Soil**	**Freshwater Sediment**
Ipconazole cc isomer	Primary	5.08	4.69
	Confirmatory	5.07	4.68
Ipconazole ct isomer	Primary	4.77	4.38
	Confirmatory	4.76	4.37

NOTE: The column eluate is diverted to waste for the first 1.8 min to prevent ionic material from the sample contaminating the mass spectrometer front plate.

MS parameters:

Instrument:	MDS Sciex 4000 Q TRAP® mass spectrometer
Ionization Mode:	Positive (+) ESI
Ion Spray Voltage:	4000 V
Scan Type:	MRM

Dwell Time: 500 milliseconds
Source Temperature: 500 °C
Curtain Gas: 20.0
Ion Source – Gas 1 / Gas 2:   50.0/50.0
Collision Gas: 8.0
Collision Cell Entrance Potential: 15.0
Resolution Q1/Q3:   Low/Low

	Primary Transition	Confirmatory Transition
Q1/Q3 Masses (amu):	334.5/70.2	336.2/70.2
Declustering Potential:	43.1	70.0
Collision Energy:	41.8	50.0
Collision Cell Exit Potential:	10.2	11.0

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.

2.11.2   Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each recovery sample set; one set prior of each to analysis of the recovery samples, and the second set of each immediately following the analysis of the recovery samples. Injection of samples and calibration standards onto the LC-MS/MS 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 fortified recovery samples. Recoveries of 70.0 to 120% (for the individual mean concentrations) are acceptable. The precision was reported in terms of the relative standard deviation (RSD) for the recovery samples and retention times. RSD values less than 20% were considered acceptable for the recovery samples (with less than 10% considered ideal). Specificity of the method was determined by examination of the control samples for peaks at the same retention times as ipconazole which might interfere

with the quantitation of the analytes. Linearity of the method was determined by the coefficient of determination (r^2), y-intercept, and slope of the regression line.

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 Section 3.0.

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 Section 3.0.

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.

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

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

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

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/L) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample mass, mL/g)
A	=	analytical result (μg/kg), concentration in the original sample

The LOD was calculated using the following equation:

$$(4) \quad LOD= (3x(SN_{ctl}))/Resp_{LS}) \text{ x } Conc_{LS} \text{ x } DF_{CNTL}$$

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
DF_{CNTL}	=	Dilution factor of the control samples (smallest dilution factor used, i.e., 10)
LOD	=	Limit of detection for the analysis

The MDL was calculated using the following equation:

$$(5) \quad MDL= MDL_{LCAL} \text{ x } DF_{CNTL}$$

where:

MDL_{LCAL}	=	The lowest concentration calibration standard (i.e., 0.00500 μg/L)
DF_{CNTL}	=	Dilution factor of the control samples (final volume of the sample divided by the original sample mass, mL/g)
MDL	=	Method detection limit reported (0.0500 μg/L $\times$ 500 = 25.0 μg/kg for ipconazole cc and 0.00350 μg/L $\times$ 500 = 1.75 μg/kg for ipconazole ct). Therefore, the MDL for ipconazole was 26.8 μg/kg.