1.0 INTRODUCTION

A method was validated on 20 to 21 May 2014 to quantify the concentrations of aldicarb, the active ingredient in Temik 15G, present in recovery samples prepared in soil. The analytical method was validated with regards to accuracy and precision, linearity, specificity, limit of quantitation (LOQ) and limit of detection (LOD). This method was validated by fortification of soil with Temik 15G at concentrations of 100, 1000 and 7000 µg/kg as aldicarb. Recovery samples were extracted twice with 0.1% formic acid in acetonitrile and were diluted into the calibration standard curve with 20:80 acetonitrile:purified reagent water (v:v) prior to analysis. Samples were then analyzed using liquid chromatography with mass spectrometry (LC/MS/MS).

The study was initiated on 12 May 2014, 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 20 to 21 May 2014 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 toxicity test followed those described in the Smithers Viscient protocol entitled "Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance in Soil" (Appendix 1).

2.2 Test and Reference Substances

2.2.1 Test Substance

The test substance, Temik 15G, was received on 10 April 2014 from Analytical and Regulatory Chemistry, Sumter, South Carolina. The following information was provided:

Name:	Temik 15G
Lot No.:	NWTEAXV179
CAS No.:	116-06-3
Purity:	14.81% as aldicarb
Expiration Date:	7 April 2016

Upon receipt at Smithers Viscient, the test substance (SMV No. 6935) was stored at room temperature in the original container in a dark, ventilated cabinet. Concentrations were adjusted for the purity of the test substance.

2.2.2 Reference Substance

The reference substance, aldicarb, was received on 21 April 2014 from Sigma Aldrich, Allentown, Pennsylvania. The following information was provided:

Name:	aldicarb
Batch No.:	SZBC166XV
CAS No.:	116-06-3
Purity:	99.9%
Expiry Date:	14 June 2017

Upon receipt at Smithers Viscient, the reference substance (SMV No. 6962) was stored at room temperature in the original container in a dark, ventilated cabinet. 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 substance, and archival of a sample of the test and reference substance is the responsibility of the Study Sponsor.

2.3 Reagents

1.	Dimethylformamide (DMF):	Burdick & Jackson, reagent grade
2.	Formic acid:	EMD, reagent grade
3.	Purified reagent water:	Prepared from a Millipore Milli-Q® Direct 8 water purification system (meets ASTM Type II requirements)
4.	Acetonitrile:	EMD, reagent grade

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:	AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source, an Acquity Sample Manager autosampler, an Acquity Binary Solvent Manager binary pump, an Acquity Column Compartment column oven, and Analyst 1.6 software for data acquisition
2.	Balance:	Mettler Toledo AG245, Mettler PJ-3000
3.	Moisture balance:	Sartorius MA-45
4.	Centrifuge:	Beckman Allegra X-12
5.	Shaker table:	VWR Standard Analog Shaker 3500 STD
6.	Laboratory equipment:	Positive displacement pipets, volumetric flasks, disposable glass vessels and pipets, Pasteur pipets, autosampler vials and amber glass bottles with Teflon®-lined cap

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

2.5 Test Soil

The soil used for the method validation was Rochester Sandy Loam soil (SMV Lot No. 021814, Sample ID 2014 100 ROCH LOAM") from Rochester, Massachusetts. Prior to testing, soil moisture content was 11.44% using a Sartorius MA-45 moisture analyzer.

2.6 Preparation of Stock Solutions

Primary stock solutions were typically prepared from the test and reference substances as summarized in the table below. All volumes can be scaled up or down as necessary. The formulation granules need to be crushed, and sonicated in a sonicator for one hour to ensure dissolution in the DMF solvent.

Primary Stock ID	Amount of Substance Weighed (g), Net Weight	Amount of Substance Weighed (g), as Active Ingredient	Final Volume (mL)	Solvent Stock	Primary Stock Concentration (mg/L)	Primary Stock Uses
Test Substance						
6935B	0.3380	0.0501	50.0	DMF	1000	Secondary stocks for recovery samples
Reference Substance						
6962-1A	0.0502	0.0501	50.0	Acetonitrile	1000	Secondary stock for calibration standards

Secondary stock solutions were prepared from the primary stock solutions as summarized in the table below.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Solvent Stock	Final Stock Concentration (mg/L)	Stock ID	Stock Uses
Test Substance							
6935B	1000	0.500	50.0	Acetonitrile	10.0	6935B-2	Low- and mid-level recovery samples
		5.00	50.0	Acetonitrile	100	6935B-3	High-level recovery samples
Reference Substance							
6962-1A	1000	0.500	50.0	Acetonitrile	10.0	6962-1A-2	Sub-stock solutions for calibration standards

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

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Solvent Stock	Final Stock Concentration (µg/L)	Stock ID	Stock Uses
Reference Substance							
6292-1A-2	10.0	0.100	10.0	Acetonitrile	100	Ana Stk 1	Calibration standards

Primary and secondary stock solutions were stored refrigerated (2 to 8 °C) in amber glass bottles fitted with Teflon®-lined caps until use. The sub-stock solutions were prepared on the day of use and discarded after use.

2.7 Mobile Phase Preparation

All volumes can be scaled up or down as necessary.

A 0.1% formic acid in purified reagent water (v:v) mobile phase solution was typically prepared by adding 1.00 mL of concentrated formic acid to 1000 mL of purified reagent water and mixed well. The mobile phase was degassed under vacuum with sonication for 10 minutes.

A 0.1% formic acid acetonitrile (v:v) mobile phase solution was typically prepared by adding 2.00 mL of concentrated formic acid to 2000 mL of acetonitrile and mixed well. The mobile phase was degassed under vacuum with sonication for 10 minutes.

A 20:80 acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 400 mL of purified reagent water with 100 mL of acetonitrile. The solution was mixed using a stir bar and stir plate.

2.8 Preparation of Calibration Standards

Calibration standards were prepared in 20:80 acetonitrile:purified reagent water (v:v) by fortifying with the 100 µg/L sub-stock solution to yield concentrations of 0.0500, 0.100, 0.500, 1.00, 1.50 and 2.00 µg/L.

2.9 Sample Fortification and Preparation

A total of 12 soil recovery samples (5.00 g dry weight) were weighed into individual 50.0-mL Nalgene® centrifuge tubes. Each concentration was prepared in triplicate with the appropriate secondary stock solution at 100, 1000 and 7000 µg/kg (dry weight), in triplicate. Three samples were left untreated and were designated as controls and were processed in the same manner as the low-level recovery samples. The fortification scheme is outlined in the table below.

Sample ID	Stock Concentration (mg/L)	Volume of Fortification (mL)	Dry weight (g)	Fortified Concentration (µg/kg)
Control D, E & F	NA[a]	NA	5.00	0.00
Low D, E & F	10.0	0.0500	5.00	100
Mid D, E & F	10.0	0.500	5.00	1000
High D, E & F	100	0.350	5.00	7000

[a] NA = Not Applicable.

2.10 Soil Extraction and Dilution

A 20.0-mL aliquot of 0.1% formic acid in acetonitrile was added to the soil recovery samples (5 g dry weight). The samples were placed on an orbital shaker table for 30 minutes at 150 rpm. The samples were then centrifuged at 3000 rpm (approximately 1200 *g*) for 10 minutes and the extracts were transferred to labeled 50.0-mL volumetric flasks. The extraction and centrifugation procedure was repeated with an additional 20.0-mL aliquot of 0.1% formic acid in acetonitrile. The second extract was combined with the first in the appropriate volumetric flasks and taken to a final volume of 50.0 mL with 0.1% formic acid in acetonitrile. Samples were further diluted into the calibration standard range with 20:80 acetonitrile:purified reagent water (v:v). The extraction and dilution procedures are detailed below. Dilution volumes can be scaled up or down as necessary. Soil masses and extraction solvent volumes can also be scaled up and down as needed but extraction efficiency will need to be evaluated to confirm method performance.

Sample ID	Fortified Concentration (µg/kg)	Dry weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Control D, E & F	0.00	5.00	20.0	50.0	0.500	10.0	200
Low D, E & F	100	5.00	20.0	50.0	0.500	10.0	200
Mid D, E & F	1000	5.00	20.0	50.0	0.125	10.0	800
High D, E & F	7000	5.00	20.0	50.0	0.100	50.0	5000

[a] Extracted and diluted with 0.1% formic acid in acetonitrile.
[b] Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v).

2.11 Analysis

2.11.1 Instrumental Conditions

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

LC parameters:

Column: XBridge C18, 2.5 µm, 2.1 mm x 50 mm (similar analysis columns may be substituted)

Mobile Phase A: 0.1% formic acid in purified reagent water

Mobile Phase B: 0.1% formic acid in acetonitrile

Gradient:

Time (min.)	Flow rate (µL/min.)	Solvent A (%)	Solvent B (%)
0.10	350	85.0%	15.0%
0.50	350	85.0%	15.0%
2.50	350	5.0%	95.0%
5.00	350	5.0%	95.0%
5.10	350	85.0%	15.0%
6.50	350	85.0%	15.0%

Injection volume: 100 µL (can be changed as necessary but must not exceed 100 µL)

Column oven: 30.0 °C

Retention Time: approximately 2.9 minutes

MS parameters:

Instrument (Other models can be used): AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source

Ionization mode: Positive

Scan type: MRM

Q1/Q3 masses: 213.10/89.00 amu

Dwell time: 200 milliseconds

Source temperature: 500 °C

Resolution Q1/Q3: Unit/Unit

Curtain Gas: 25.00

Ion Source Gas 1/Gas 2: 70.00/70.00

Ion Spray Voltage: 5500

Collision Gas: 10.00

Declustering Potential: 100.00

Entrance Potential: 3.00

Collision Energy: 24.00

Collision Cell Exit Potential: 11.00

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 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 low-, mid- and high-level recovery samples. The precision was reported in terms of the standard deviation and relative standard deviation for the retention time and the percent recovery values of the low-, mid- and high-level recovery samples. Specificity of the method was determined by examination of the control samples for peaks at the same retention time as aldicarb, the active ingredient in Temik 15G which might interfere with the quantitation of the analytes. A polynomial calibration curve was used for this testing due to the nature of the LC/MS/MS detection. This method was evaluated based on the correlation coefficient (r^2) and the recoveries of the calibration standards.

2.13 Limit of Detection and Quantitation

The limit of quantitation (LOQ) was calculated from the lowest concentration calibration standard and the dilution factor of the control samples. The limit of detection (LOD) was calculated using three times the signal-to-noise value of the control samples.

3.0 Calculations

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

$$(1) \qquad y = ax^2 + bx + c$$

$$(2) \qquad DC(x) = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

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

where:

y	=	detector response (peak area) for analyte
a, b and c	=	regression constants
DC (x)	=	detected concentration (µg/L) in the sample
C	=	constant c minus the peak area; C = (c - y)
DF	=	dilution factor (the final sample volume divided by the original sample mass)
A	=	concentration of the analyte in the original sample

The limit of quantitation (LOQ) was calculated using the following equation:

$$(4)\ LOQ_{INST} = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

$$(5)\ LOQ = LOQ_{INST} \times DF_{CTRL}$$

where:

$Area_{LS}$	=	mean detector response (peak area) of the low concentration calibration standard (two injections)
a, b, c	=	regression constants
C	=	regression constant ; C = (c - $Area_{LS}$)
LOQ_{INST}	=	limit of quantitation on the instrument
DF_{CTRL}	=	dilution factor of the control samples (smallest dilution factor used)
LOQ	=	limit of quantitation reported for the analysis

The limit of detection (LOD) was determined by calculating the average noise level in chromatograms of purified reagent water control solutions and comparing them to the signal of a lowest calibration standard of known concentration. Three chromatograms were examined for noise-related peaks near the retention time of the analyte during the method validation. The LOD was calculated as 3 times the concentration equivalent of the mean noise level.