1.0 INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of novaluron and its degradates, CLA and CPU, in soil matrices. The method was validated (15 November 2017 to 18 January 2018) to quantify the concentrations of novaluron and its degradates, CLA and CPU, present in recovery samples prepared in artificial sediment and loamy sand. 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 in artificial sediment and loamy sand by fortification with novaluron and its degradates, CLA and CPU, at concentrations of 50.0 (LOQ) and 500 (10X LOQ) µg/kg. Recovery samples were extracted with methanol diluted with 50/50 acetonitrile/purified reagent water (v/v). The 10X LOQ recovery samples were further diluted into the calibration range with 50/50 acetonitrile/purified reagent water (v/v). All samples were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

The study was initiated on 24 April 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 15 November 2017 to 22 January 2018 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 Novaluron and its Degradates in Soil Matrices 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/825/00 rev. 8.1 (EC, 2010) and OCSPP 850.6100 (U.S. EPA, 2012).

2.2 Test Substances

The test substance, novaluron technical, was received on 9 January 2017 from ADAMA Makhteshim Ltd., Beer-Sheva, Israel. The following information was provided:

Name:	novaluron technical
Lot No.:	96869065
CAS No.:	116714-46-6
Purity:	98.8% (Certificate of Analysis, Appendix 2)
Recertification Date:	1 March 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8690) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, CPU (novaluron degradate), was received on 7 April 2017 from ADAMA Makhteshim Ltd., Beer-Sheva, Israel. The following information was provided:

Name:	CPU (novaluron degradate)
Synonym:	1-[-3-chloro-4-(1,1,2-trifluoro-2 trifluromethoxyethoxy)phenyl]urea
Lot No.:	554-187-04
CAS No.:	Not Listed
Purity:	86.9% (Certificate of Analysis, Appendix 2)
Recertification Date:	7 June 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8853) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, CLA (novaluron degradate), was received on 9 January 2017 from ADAMA Makhteshim Ltd., Beer-Sheva, Israel. The following information was provided:

Name:	CLA (novaluron degradate)
Synonym:	3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)aniline
Batch No.:	554-136-01
CAS No.:	Not Listed
Purity:	98.9% (Certificate of Analysis, Appendix 2)
Recertification Date:	3 March 2019

Upon receipt at Smithers Viscient, the test substance (SMV No. 8692) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

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

2.3 Reagents

1.	0.1% Formic acid in water:	Fisher Chemical, reagent grade
2.	0.1% Formic acid in acetonitrile:	Fisher Chemical, reagent grade
3.	Methanol:	EMD, reagent grade
4.	Acetonitrile:	EMD, reagent grade
5.	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.	Instrument:	Sciex 6500+ QTRAP mass spectrometer equipped with an Sciex IonDrive Turbo V ion source Shimadzu SIL-20ACXR autosampler Shimadzu DGU-20A5R vacuum degassers Shimadzu LC-20ADXR binary pumps Shimadzu CTO-20AC column oven Shimadzu CBM-20A communications bus Analyst 1.6.3 software for data acquisition
2.	Balances:	Mettler Toledo PG-2002-S; Mettler Toledo PJ-3000; O'Haus EX4202/E
3.	Moisture Balances:	Mettler Toledo HB43-S; Sartorius MA-150

4.	Shaker Tables:	Orbit 3520; VWR 3500STD
5.	Centrifuges:	Beckman 367160; Beckman Allegra X-12; Thermo Scientific Sorvall Legend XFR
6.	Laboratory equipment:	Positive displacement pipets, volumetric flasks, disposable glass vials, disposable glass pipets, Teflon centrifuge tubes, graduated cylinders, Pasteur pipets, autosampler vials, and amber glass bottles with Teflon-lined cap

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 matrices used during this method validation were artificial sediment and loamy sand.

The soils used for the method validation were artificial sediment characterized as sandy loam (SMV Lot Nos. 060517 and 060717) and loamy sand soil (SMV Lot No. 041917b) from Sunnynook Farm in Rochester, Massachusetts. Soil was characterized by Agvise Laboratories, Northwood, North Dakota and the characterization data are listed in the table below.

Soil Type	% Sand, Silt, Clay	Bulk Density (gm/cc)	CEC[a] (meq/100 g)	% Organic Matter (Walkley Black)	pH in 1/1 soil/water Ratio
Artificial Sediment (sandy loam)	78, 6, 16	1.02	7.9	2.1	7.6
Loamy Sand	83, 16, 1	0.96	13.6	13.5	6.6

[a] CEC = Caption Exchange Capacity

2.6 Preparation of Liquid Reagent 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 typically prepared by combining 500 mL of acetonitrile and 500 mL of purified reagent water. The solution was mixed well 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 typically prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water.

2.7 Preparation of Stock Solutions

The volumes and masses listed in this section are representative of the stocks prepared during testing, but may not reflect the exact quantities for each separate validation. Volumes and masses may be changed; 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
8690W	0.05060	0.04999	Acetonitrile	50.0	1000	Secondary stock solutions
8853AS	0.05774	0.05018	Acetonitrile	50.0	1000	Secondary stock solutions
8692Z	0.05072	0.05016	Acetonitrile	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
8690W	1000	0.500	50.0	Acetonitrile	8690W-1	10.0	Sub-stock solutions
	1000	5.00	50.0		8690W-2	100	Sub-stock solutions
8853AS	1000	0.500	50.0	Acetonitrile	8853AS-1	10.0	Sub-stock solutions
	1000	5.00	50.0		8853AS-2	100	Sub-stock solutions
8692Z	1000	0.500	50.0	Acetonitrile	8692Z-1	10.0	Sub-stock solutions
	1000	5.00	50.0		8692Z-2	100	Sub-stock solutions

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
8690W-2	100	1.00	10.0	Acetonitrile	Tech Mix Stk 1	10.0	LOQ and 10X LOQ recovery samples
8853AS-2		1.00					
8692Z-2		1.00					
8690W-1	10.0	0.0500	50.0	Acetonitrile	Ana Mix Stk 1	0.0100	Calibration standards
8853AS-1		0.0500					
8692Z-1		0.0500					

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

The effects of matrix enhancement or suppression were evaluated through the assessment of matrix-matched and solvent-based calibration standards in the following manner. Two sets of calibration standards were prepared. One set was prepared in 50/50 acetonitrile/purified reagent

water (v/v) and a second set was prepared in a matrix blank sample (see Section 2.9 for additional information). Both sets of calibration standards were prepared in the same manner by fortifying with the 0.0100 mg/L mixed sub-stock solution to yield concentrations of 0.0100, 0.0200, 0.0500, 0.100, 0.150, and 0.200 µg/L. This procedure is detailed in the table below.

Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Standard Concentration (µg/L)	Sample ID[a]
Ana Mix Stk 1	0.0100	0.0200	20.0	0.0100	Std 1
		0.0200	10.0	0.0200	Std 2
		0.0500	10.0	0.0500	Std 3
		0.100	10.0	0.100	Std 4
		0.150	10.0	0.150	Std 5
		0.200	10.0	0.200	Std 6

[a] The Sample IDs presented are for the solvent-based calibration standards. Matrix-matched calibration standards were prepared per the table above and were labeled M Std 1, M Std 2, M Std 3, M Std 4, M Std 5, and M Std 6.

2.9 Sample Fortification and Preparation

For artificial sediment (CPU and CLA) and loamy sand, 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 mixed sub-stock solution at concentrations of 50.0 (LOQ) and 500 (10X LOQ) µg/kg (dry weight). For artificial sediment (novaluron), 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 secondary stock solution at concentrations of 50.0 (LOQ) and 500 (10X LOQ) µg/kg (dry weight). Five replicates were produced for each concentration level. Two samples per matrix were left unfortified to serve as controls and were diluted in the same fashion as the LOQ concentration recovery samples. In addition, two reagent blanks (no test substance or matrix) and one matrix blank were prepared and processed in the same manner as the control samples. The dosing procedure is outlined in the tables below.

Artificial Sediment (CPU and CLA) and Loamy Sand Recovery Samples

Sample ID 14125-6101-	Sample Type	Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Fortified Concentration (µg/kg)
01	Matrix Blank	NA[a]	NA	5.00	0.00
02 & 03	Reagent Blank	NA	NA	0.00	0.00
04 & 05	Control	NA	NA	5.00	0.00
06, 07, 08, 09, & 10	LOQ	10.0	0.0250	5.00	50.0
11, 12, 13, 14, & 15	10X LOQ	10.0	0.250	5.00	500

[a] NA = Not Applicable

Artificial Sediment (Novaluron) Recovery Samples

Sample ID 14125-6101-	Sample Type	Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Weight (g)	Fortified Concentration (µg/kg)
16	Matrix Blank	NA[a]	NA	5.00	0.00
17 & 18	Reagent Blank	NA	NA	0.00	0.00
19 & 20	Control	NA	NA	5.00	0.00
21, 22, 23, 24, & 25	LOQ	10.0	0.0250	5.00	50.0
26, 27, 28, 29, & 30	10X LOQ	10.0	0.250	5.00	500

[a] NA = Not Applicable

2.10 Sample Extraction

A 20-mL aliquot of methanol was added to each soil recovery sample (5.00 g dry weight) and they were placed on a shaker table for 30 minutes at 150 rpm. Samples were then centrifuged at 3000 rpm for 10 minutes and the extracts were transferred to 50-mL volumetric flasks. The extraction and centrifugation procedures were repeated with an additional 20-mL aliquot of methanol. The extracts were combined, taken to volume (50.0 mL) with methanol, and mixed well. An aliquot of each sample was then diluted with 50/50 acetonitrile/purified reagent water (v/v) and mixed well. The 10X LOQ recovery sample extracts were further diluted into the calibration standard range with 50/50 acetonitrile/purified reagent water (v/v). Samples were

then centrifuged at 13,000 rpm for 10 minutes. The extraction and dilution procedures are detailed in the tables below.

Artificial Sediment (CPU and CLA) and Loamy Sand Recovery Samples

Sample ID 14125-6101-	Sample Type	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Tertiary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
01	Matrix Blank	0.00	5.00	20.0	50.0	2.00	100	NA[c]	NA	500
02 & 03	Reagent Blank	0.00	0.00	20.0	50.0	0 200	10.0	NA	NA	500
04 & 05	Control	0.00	5.00	20.0	50.0	0 200	10.0	NA	NA	500
06, 07, 08, 09, & 10	LOQ	50.0	5.00	20.0	50.0	0 200	10.0	NA	NA	500
11, 12, 13, 14, & 15	10X LOQ	500	5.00	20.0	50.0	0 200	10.0	1.00	10.0	5000

[a] Extraction and dilution solvent: methanol
[b] Dilution solvent: 50/50 acetonitrile/purified reagent water (v/v)
[c] NA = Not Applicable

Artificial Sediment (Novaluron) Recovery Samples

Sample ID 14125-6101-	Sample Type	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Tertiary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
16	Matrix Blank	0.00	5.00	20.0	50.0	2.00	100	NA[c]	NA	500
17 & 18	Reagent Blank	0.00	0.00	20.0	50.0	0 200	10.0	NA	NA	500
19 & 20	Control	0.00	5.00	20.0	50.0	0 200	10.0	NA	NA	500
21, 22, 23, 24, & 25	LOQ	50.0	5.00	20.0	50.0	0 200	10.0	NA	NA	500
26, 27, 28, 29, & 30	10X LOQ	500	5.00	20.0	50.0	0 200	10.0	1.00	10.0	5000

[a] Extraction and dilution solvent: methanol
[b] Dilution solvent: 50/50 acetonitrile/purified reagent water (v/v)
[c] NA = Not Applicable

2.11 Analysis

2.11.1 Instrumental Conditions

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

CPU and CLA in Artificial Sediment

LC parameters:

Column:	Waters XBridge BEH C18, 2.5 µm, 2.1 × 50 mm
Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	0.1% formic acid in acetonitrile

Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.300	70.0	30.0
0.50	0.300	70.0	30.0
1.50	0.300	40.0	60.0
4.00	0.300	0.00	100
5.00	0.300	0.00	100
5.10	0.300	70.0	30.0
6.10	0.300	70.0	30.0

Run time:	6.1 minutes
Injector Rinse solvent:	30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Column temperature:	40 °C
Sample temperature:	10 °C
Injection volume:	20 µL
Retention Times:	Approximately 3.0 minutes for CPU Approximately 3.3 minutes for CLA

MS parameters:

Instrument:	Sciex 6500+ QTRAP mass spectrometer
Ionization Mode:	Positive (+) ESI
Ion Spray Voltage:	5000 V
Scan type:	MRM
Source Temperature:	500 °C
Curtain Gas:	25.00
Ion Source – Gas 1 / Gas 2:	20.00 / 10.00
Collision Gas:	Medium
Collision Cell Entrance Potential:	10.00
Resolution (Q1/Q3):	Unit/Unit

Novaluron in Artificial Sediment

LC parameters:

Column: Waters XBridge BEH C18, 2.5 µm, 2.1 × 50 mm
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
1.00	0.400	60.0	40.0
1.10	0.400	20.0	80.0
3.00	0.400	0.00	100
4.60	0.400	0.00	100
4.70	0.400	60.0	40.0
6.00	0.400	60.0	40.0

Run time: 6.0 minutes
Injector Rinse solvent: 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Column temperature: 40 °C
Sample temperature: 10 °C
Injection volume: 25.0 µL
Retention Times: Approximately 2.5 minutes for novaluron

MS parameters:

Instrument: Sciex 6500+ QTRAP mass spectrometer
Ionization Mode: Positive (+) ESI
Ion Spray Voltage: 5500 V
Scan type: MRM
Source Temperature: 550 °C
Curtain Gas: 25.00
Ion Source – Gas 1 / Gas 2: 60.00 / 60.00
Collision Gas: High
Collision Cell Entrance Potential: 10.00
Resolution (Q1/Q3): Unit/Unit

Novaluron, CPU, and CLA in Loamy Sand

LC parameters:

Column: Waters XBridge BEH C18, 2.5 µm, 2.1 × 50 mm
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile

Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
1.00	0.400	60.0	40.0
1.10	0.400	20.0	80.0
3.00	0.400	0.00	100
4.60	0.400	0.00	100
4.70	0.400	60.0	40.0
6.00	0.400	60.0	40.0

Run time: 6.0 minutes
Injector Rinse solvent: 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Column temperature: 40 °C
Sample temperature: 10 °C
Injection volume: 25.0 µL
Retention Times: Approximately 2.4 minutes for novaluron
Approximately 2.1 minutes for CPU
Approximately 2.2 minutes for CLA

MS parameters:

Instrument: Sciex 6500+ QTRAP mass spectrometer
Ionization Mode: Positive (+) ESI
Ion Spray Voltage: 5500 V
Scan type: MRM
Source Temperature: 550 °C
Curtain Gas: 25.00
Ion Source – Gas 1 / Gas 2: 60.00 / 60.00
Collision Gas: High
Collision Cell Entrance Potential: 10.00
Resolution (Q1/Q3): Unit/Unit

Matrix	Analyte	Analysis	Q1/Q3 Mass (amu/amu)	Dwell Time (milliseconds)	Declustering Potential	Collision Energy	Collision Cell Exit Potential
Artificial Sediment	Novaluron	Primary	493 1/158.0	50.0	81.0	31.0	10.0
		Confirmatory	493 1/140.9	50.0	81.0	65.0	12.0
	CPU	Primary	353.0/275.2	50.0	91.0	60.0	20.0
		Confirmatory	353.0/108.1	50.0	91.0	40.0	12.0
	CLA	Primary	310 1/108.0	50.0	86.0	45.0	18.0
		Confirmatory	310 1/127.2	50.0	86.0	41.0	10.0
Loamy Sand	Novaluron	Primary	493.1/158.0	65.0	81.0	31.0	10.0
		Confirmatory	493.1/140.9	65.0	81.0	65.0	12.0
	CPU	Primary	353.0/275.0	65.0	50.0	37.0	28.0
		Confirmatory	353.0/309.9	65.0	50.0	31.0	30.0
	CLA	Primary	310.1/108.0	65.0	86.0	45.0	18.0
		Confirmatory	310.1/127.2	65.0	86.0	41.0	10.0

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 for both matrix-matched and solvent-based standards (for four sets in total) were analyzed with each recovery sample set. Calibration standards were interspersed among analysis of the recovery samples, every two to six injections. 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 to 110% (for the mean recovery at each fortification level) are acceptable. The precision was reported in terms of the relative standard deviation (RSD) for the recovery samples. RSD values less than 20% were considered acceptable for the recovery samples.

Specificity of the method was determined by examination of the control samples for peaks at the same retention times as novaluron and its degradates, CPU and CLA, 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, and linearity extended over a range appropriate to 30% of the LOQ to 20% above the highest level.

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.

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

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

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

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 volume)
A	=	analytical result (µg/L), concentration in the original sample

The LOD was calculated using the following equation:

$$(4) \qquad LOD = (3\times(N_{ctl}))/Resp_{LS}) \times Conc_{LS} \times DF_{CNTL}$$

where:

SN_{ctl}	=	mean 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., 500)
LOD	=	limit of detection for the analysis

The method detection limit (MDL) is defined as the lowest concentration that can be detected by this method in test solution samples. The MDL is calculated (Equation 5) based on the concentration of the low calibration standard and the dilution factor of the control samples.

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

where:

MDL_{LCAL}	=	lowest concentration calibration standard (0.0100 µg/L)
DF_{CNTL}	=	dilution factor of the control samples (smallest dilution factor used, 500)
MDL	=	method detection limit reported for the analysis (0.0100 µg/L × 500 = 5.00 µg/L)

APPENDIX 3 - METHOD FLOW CHART

Extract samples with a 20-mL aliquot of methanol and place on a shaker table at 150 rpm for 30 minutes

↓

Centrifuge samples at 3000 rpm for 10 minutes and transfer extracts to 50-mL volumetric flasks

↓

Repeat with 20.0 mL of methanol and place on a shaker table at 150 rpm for 30 minutes

↓

Combine the two extracts, adjusting the volume to 50.0 mL with the extraction solvent

↓

Dilute the extracts with 50/50 acetonitrile/purified reagent water (v/v), with 10X LOQ samples further diluted into the calibration standard range with 50/50 acetonitrile/purified reagent water (v/v)

↓

Centrifuge at 13,000 rpm for 10 minutes

↓

Place in autosampler vials

↓

Analyse by LC-MS/MS