1.0 INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of triallate and TCPSA in two different soil types, clay loam 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 by fortification of soil with triallate and TCPSA at concentrations of 50.0 (limit of quantitation, LOQ) and 500 µg/kg (10 × LOQ). All recovery samples were extracted with 50:50 acetonitrile:purified reagent water (v:v). The triallate recovery samples were diluted into the calibration standard range with 50:50 acetonitrile:purified reagent water (v:v). The TCPSA recovery samples were diluted into the calibration standard range with acetonitrile. All samples were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

The study was initiated on 2 August 2016, 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 17 August to 1 September 2016 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 Triallate and TCPSA 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, 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

The test substance, triallate AS, was received on 9 June 2016 from Chem Service, Westchester, Pennsylvania. The following information was provided:

Name:	triallate AS
Lot No.:	5125900
CAS No.:	2303-17-5
Purity:	99.5% (Certificate of Analysis, Appendix 2)
Recertification Date:	17 June 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8323) 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, TCPSA, was received on 7 June 2016 from EPL Archives, Inc., Sterling, Virginia. The following information was provided:

Name:	TCPSA
Synonym:	sodium 2,3,3-trichloro-2-propene-1-sulfonate
Batch No.:	SP15-106-1-1
CAS No.:	[65600-61-5]
Purity:	99.8% (Certificate of Analysis, Appendix 2)
Expiration Date:	16 June 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8303) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

For both test substances, determination of stability and characterization, verification of identity, maintenance of records on the test substances, and archival of a sample of each of the test substances are the responsibility of the Study Sponsor.

2.3 Reagents

1. Acetonitrile:	EMD, reagent grade
2. 0.1% Formic acid in water:	EMD, reagent grade; Fisher Chemical, reagent grade
3. 0.1% Formic acid in acetonitrile:	Fisher Chemical, reagent grade
4. Formic acid:	EMD, reagent grade
5. Methanol:	EMD, reagent grade
6. 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 Instrumentation and Laboratory Equipment

1. Instrument:	AB Sciex API 5000 mass spectrometer equipped with an ESI Turbo V source Waters Acquity Binary Solvent Manager Waters Acquity Sample Manager – FTN Waters Acquity Column Compartment Analyst version 1.6 software for data acquisition
2. Balances:	Mettler PG-2002-S, Mettler Toledo XSE205DU
3. Centrifuge:	Beckman Allegra X-12, Eppendorf 5417C
4. Shaker table:	VWR 3500
5. Moisture balance:	Sartorius Moisture Analyzer MA-45
6. 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, low-binding centrifuge tubes, 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 Matrices

The soils used for the method validation were clay loam soil
(SMV Lot No. 091215AS-DU-L 0-6") from Eastern, North Dakota, and Rochester loamy sand soil (SMV Lot No. 012616A) from Rochester, Massachusetts. Prior to testing, soil moisture content was determined to be 14.35% for the clay loam soil and 19.52% for the Rochester loamy sand 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
Clay Loam	44, 26, 30	0.99	20.2	5.2	5.4
Loamy Sand	78, 18, 4	1.06	9.7	4.9	6.8

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 50:50 acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 500 mL of acetonitrile with 500 mL of purified reagent water. 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 typically prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water.

A 90:10 purified reagent water: acetonitrile (v:v) autosampler purge wash solution was typically prepared by combining 1800 mL of purified reagent water and 200 mL of acetonitrile.

A 0.1% formic acid in acetonitrile liquid reagent solution was typically prepared by adding 2.00 mL of formic acid to 2000 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 0.1% formic acid in purified reagent water mobile phase solution was typically prepared by adding 2.00 mL of formic acid to 2000 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.

2.7 Preparation of Stock Solutions

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

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
8323-1E	0.0505	0.0502	Acetonitrile	50.0	1000	Secondary stock solution
8303-1C	0.01004	0.01002	50:50 acetonitrile: purified reagent water	100	100	Sub-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
8323-1E	1000	5.00	50.0	Acetonitrile	8323-1E-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
8323-1E-2	100	1.00	10.0	Acetonitrile	Mix-Stk 1	10.0	High-level recovery samples

8303-1C	100	1.00					and sub-stock solution
Mix-Stk 1	10.0	1.00	10.0		Mix-Stk 2	1.00	LOQ recovery samples, and sub-stock solution
Mix-Stk 2	1.00	0.100	10.0		Mix-Stk 3	0.0100	Calibration Standards and sub-stock solution
Mix-Stk 3	0.0100	1.00	10.0		Mix-Stk 4	0.00100	Calibration Standards

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

Validation with Triallate in clay loam and loamy sand soils

Calibration standards were prepared in 50:50 acetonitrile:purified reagent water (v:v) by fortifying with the 0.00100 or 0.0100 mg/L mixed test substance sub-stock solution to yield test substance concentrations of 0.00500, 0.0100, 0.0250, 0.0500, 0.100, 0.250, and 0.500 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
Mix-Stk 4	0.00100	0.0500	10.0	0.00500	Std 1
		0.100	10.0	0.0100	Std 2
Mix-Stk 3	0.0100	0.0250	10.0	0.0250	Std 3
		0.0500	10.0	0.0500	Std 4
		0.100	10.0	0.100	Std 5
		0.250	10.0	0.250	Std 6
		0.500	10.0	0.500	Std 7

Validation with TCPSA in clay loam and loamy sand soils

Calibration standards were prepared in acetonitrile by fortifying with the 0.00100 or 0.0100 mg/L mixed test substance sub-stock solution to yield test substance concentrations of 0.00500, 0.0100, 0.0250, 0.0500, 0.100, 0.250, and 0.500 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
Mix-Stk 4	0.00100	0.0500	10.0	0.00500	Std 8
		0.100	10.0	0.0100	Std 9
Mix-Stk 3	0.0100	0.0250	10.0	0.0250	Std 10
		0.0500	10.0	0.0500	Std 11
		0.100	10.0	0.100	Std 12
		0.250	10.0	0.250	Std 13
		0.500	10.0	0.500	Std 14

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 (the LOQ). Calibration standards used to assess possible matrix effects were prepared as follows by fortifying with the 0.0100 mg/L mixed test substance sub-stock solution to yield test substance concentrations of 0.0500 µg/L for triallate and TCPSA.

2.8.2.1 Matrix-Matched Standards

Prepared in soil final fraction extract for triallate analysis

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID[b]
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	MM-Std A
		0.0100	2.00	0.0500	MM-Std B
		0.0100	2.00	0.0500	MM-Std C
		0.0100	2.00	0.0500	MM-Std G
		0.0100	2.00	0.0500	MM-Std H
		0.0100	2.00	0.0500	MM-Std K

[a] Samples were diluted with the final fraction of the Control A-1 for the clay loam soil or Control C-1 for the loamy sand soil following dilution in 50:50 acetonitrile:purified reagent water (v:v; see Section 2.10 for extract preparation and dilution procedures).

[b] Sample ID codes included: A, B, and C (triallate analysis for clay loam soil); G, H, and K (triallate analysis for loamy sand).

Prepared in soil final fraction extract for TCPSA analysis

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID[b]
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	MM-Std D
		0.0100	2.00	0.0500	MM-Std E
		0.0100	2.00	0.0500	MM-Std F
		0.0100	2.00	0.0500	MM-Std L
		0.0100	2.00	0.0500	MM-Std M
		0.0100	2.00	0.0500	MM-Std N

[a] Samples were diluted with the final fraction of the Control A-2 for the clay loam soil or Control C-2 for the loamy sand soil following dilution in 100% ACN (v:v; see Section 2.10 for extract preparation and dilution procedures).

[b] Sample ID codes included: D, E, and F (TCPSA analysis for clay loam soil); and L, M, and N (TCPSA analysis for loamy sand).

2.8.2.2 Non Matrix-Matched Standards

For triallate analysis:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	Std A
		0.0100	2.00	0.0500	Std B
		0.0100	2.00	0.0500	Std C
		0.0100	2.00	0.0500	Std G
		0.0100	2.00	0.0500	Std H
		0.0100	2.00	0.0500	Std K

[a] Samples were diluted with 50:50 acetonitrile:purified reagent water (v:v).

For TCPSA analysis:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)[a]	Standard Concentration (µg/L)	Sample ID
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	Std D
		0.0100	2.00	0.0500	Std E
		0.0100	2.00	0.0500	Std F
		0.0100	2.00	0.0500	Std L
		0.0100	2.00	0.0500	Std M
		0.0100	2.00	0.0500	Std N

[a] Samples were diluted with acetonitrile.

2.9 Sample Fortification and Preparation

The recovery samples were prepared in two different matrices (clay loam soil and loamy sand soil) with triallate and TCPSA 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 Nalgene® 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 clay loam soil:

Sample ID	Sub-Stock Concentration (mg/L)	Fortification Volume (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	1.00	0.250	5.00	50.0
High A, B, C, D, & E	10.0	0.250	5.00	500

[a] NA = Not Applicable.

Recovery samples in loamy sand soil:

Sample ID	Sub-Stock Concentration (mg/L)	Fortification Volume (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	1.00	0.250	5.00	50.0
High F, G, H, I, & J	10.0	0.250	5.00	500

[a] NA = Not Applicable.

2.10 Soil Extraction

A 20.0-mL aliquot of 50:50 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 50:50 acetonitrile:purified reagent water (v:v). The extracts were combined, taken to volume (50.0 mL) with 50:50 acetonitrile:purified reagent water (v:v) and mixed well. The triallate recovery sample extracts were further diluted into the calibration standard range with 50:50 acetonitrile:purified reagent water (v:v). These samples were labeled as "-1". For the TCPSA recovery analysis, the samples were further diluted into the calibration standard range with acetonitrile. These samples were labeled as "-2". Prior to analysis all samples were centrifuged at 13,000 rpm for five minutes using low-binding

centrifuge tubes. All recovery samples were transferred to HPLC vials for analysis via LC-MS/MS. Secondary dilution volumes can be scaled up or down as necessary. The extraction and dilution procedures are detailed below.

2.10.1 Triallate

For triallate analysis in clay loam soil

Sample ID	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[b] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-1-1	0.00	NA[c]	20.0	50.0	0.100	10.0	1000
Control A-1	0.00	5.00	20.0	50.0	0.100	10.0	1000
Control B-1	0.00	5.00	20.0	50.0	0.100	10.0	1000
LOQ A-1, B-1, C-1, D-1, & E-1	50.0	5.00	20.0	50.0	0.100	10.0	1000
High A-1, B-1, C-1, D-1, & E-1	500	5.00	20.0	50.0	0.0200	20.0	10,000

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

For triallate analysis in loamy sand soil

Sample ID	Nominal Concentration (µg/kg)	Dry Weight (g)	Extract Volume[a] (mL)	Final Volume[b] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-2-1	0.00	NA[c]	20.0	50.0	0.100	10.0	1000
Control C-1	0.00	5.00	20.0	50.0	0.100	10.0	1000
Control D-1	0.00	5.00	20.0	50.0	0.100	10.0	1000
LOQ F-1, G-1, H-1, I-1, & J-1	50.0	5.00	20.0	50.0	0.100	10.0	1000
High F-1, G-1, H-1, I-1, & J-1	500	5.00	20.0	50.0	0.0200	20.0	10,000

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

2.10.2 TCPSA

For TCPSA analysis in clay loam 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-1-2	0.00	NA[c]	20.0	50.0	0.100	10.0	1000
Control A-2	0.00	5.00	20.0	50.0	0.100	10.0	1000
Control B-2	0.00	5.00	20.0	50.0	0.100	10.0	1000
LOQ A-2, B-2, C-2, D-2, & E-2	50.0	5.00	20.0	50.0	0.100	10.0	1000
High A-2, B-2, C-2, D-2, & E-2	500	5.00	20.0	50.0	0.0200	20.0	10,000

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

For TCPSA analysis in in 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-2-2	0.00	NA[c]	20.0	50.0	0.100	10.0	1000
Control C-2	0.00	5.00	20.0	50.0	0.100	10.0	1000
Control D-2	0.00	5.00	20.0	50.0	0.100	10.0	1000
LOQ F-2, G-2, H-2, I-2, & J-2	50.0	5.00	20.0	50.0	0.100	10.0	1000
High F-2, G-2, H-2, I-2, & J-2	500	5.00	20.0	50.0	0.0200	20.0	10,000

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

2.11 Analysis

2.11.1 Instrumental Conditions

Validation with Triallate

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

LC Parameters:

Column:	XBridge 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 (%)
0.00	0.350	75.0	25.0
0.50	0.350	75.0	25.0
4.00	0.350	0.0	100
6.00	0.350	0.0	100
6.10	0.350	75.0	25.0
7.50	0.350	75.0	25.0

Run Time:	7.5 minutes
Injector Rinse Solvent 1:	30:30:40 acetonitrile:methanol:purified reagent water (v:v:v)
Injector Rinse Solvent 2:	90:10 purified reagent water:acetonitrile (v:v:v)
Column Temperature:	40 °C
Sample Temperature:	5 °C
Injection Volume:	100 µL

MS Parameters:

Instrument:	AB Sciex API 5000 mass spectrometer equipped with an ESI Turbo V source
Ionization Mode:	Positive (+) ESI
Ion Spray Voltage:	5000 V
Scan Type:	MRM
Dwell Time:	500 milliseconds
Resolution Q1/Q3:	Unit/Unit
Source Temperature:	500 °C
Curtain Gas:	30.00
Ion Source – Gas 1/Gas 2:	30.00/30.00
Collision Gas:	4.00
Collision Cell Entrance Potential:	10.00
Collision Cell Exit Potential:	15.00

Declustering Potential: 50.00

Matrix	Analysis	Retention Time	Q1/Q3 Mass (amu/amu)	Collision Energy
Clay loam	Primary	3.86	304.1 / 86.1	24.70
	Confirmatory	3.85	304.1 / 142.8	41.00
Loamy sand	Primary	3.86	304.1 / 86.1	24.70
	Confirmatory	3.85	304.1 / 142.8	41.00

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.

Validation with TCPSA

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

LC Parameters:

Column: Atlantis® HILIC silica, 3 µm, 3.0 × 100 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 (%)
0.00	0.200	10.0	90.0
5.00	0.200	10.0	90.0

Run Time: 5.0 minutes
Injector Rinse Solvent 1: 30:30:40 acetonitrile:methanol:purified reagent water (v:v:v)
Injector Rinse Solvent 2: 90:10 purified reagent water:acetonitrile (v:v:v)
Column Temperature: 40 °C
Sample Temperature: 5 °C
Injection Volume: 50.0 µL

MS Parameters:

Instrument: AB Sciex API 5000 mass spectrometer equipped with an ESI Turbo V source
Ionization Mode: Negative (-) ESI
Ion Spray Voltage: -4500 V
Scan Type: MRM
Dwell Time: 800 milliseconds
Resolution Q1/Q3: Unit/Unit

Source Temperature:	500 °C
Curtain Gas:	30.00
Ion Source – Gas 1/Gas 2:	30.00/30.00
Collision Gas:	4.00
Collision Cell Entrance Potential:	-10.00
Collision Cell Exit Potential:	-15.00
Declustering Potential:	-50.00

Matrix	Analysis	Retention Time	Q1/Q3 Mass (amu/amu)	Collision Energy
Clay loam	Primary	3.95	224.8 / 79.8	-50.00
	Confirmatory	3.94	222.8 / 186.9	-19.40
Loamy sand	Primary	3.95	224.8 / 79.8	-50.00
	Confirmatory	3.94	222.8 / 186.9	-19.40

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 to analysis of the recovery samples, and the second set 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 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 and RSD values less than 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 times as triallate and TCPSA which might interfere with the quantitation of the

analytes. A 1/x weighted linear regression calibration curve was used for this testing. This calibration curve was evaluated based on the correlation coefficient (r), the coefficient of determination (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 (50.0 µg/kg). 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 with 1/x weighting analysis (using Analyst 1.6), 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 volume)
A	=	analytical result (µg/kg), concentration in the original sample

The LOD was calculated using the following equation:

$$LOD = (3x(SN_{ctl}))/Resp_{LS} \times Conc_{LS}$$

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.

$$(4) \quad MDL = MDL_{LCAL} \text{ x } DF_{CNTL}$$

where:

MDL_{LCAL}	=	the lowest concentration calibration standard (0.00500 µg/L)
DF_{CNTL}	=	dilution factor of the control samples (smallest dilution factor used 1000)
MDL	=	method detection limit reported for the analysis of triallate, or TCPSA recovery samples (5.00 µg/kg)