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 aqueous matrices, ground water and surface water. 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 aqueous matrices with triallate and TCPSA at concentrations of 0.100 (limit of quantitation, LOQ) and 1.00 µg/L (10 × LOQ). Recovery samples were diluted with acetonitrile followed by dilution into the calibration standard range with 50:50 acetonitrile:purified reagent water (v:v) for triallate and TCPSA primary analysis (T3 column) samples and with 90:10 acetonitrile:purified reagent water (v:v) for TCPSA confirmatory (HILIC column) analysis samples, respectively. 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 19 August to 20 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 Aqueous 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% (Certificates 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.

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

2.3 Reagents

1. Acetonitrile:	EMD, reagent grade
2. 0.1% Formic acid in water:	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 (for triallate analysis and TCPSA confirmatory analysis):
   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

   Instrument (for TCPSA primary analysis):
   AB Sciex API 5000 mass spectrometer equipped with an ESI Turbo V source
   Shimadzu 20AD/LC-20AD Solvent delivery pumps
   Shimadzu 20AD/SIL-20ACHT autoinjector
   Shimadzu 20AD/CTO-20A column compartment
   Analyst version 1.4.2 software for data acquisition
2. Balances: Mettler Toledo XSE205DU
3. Centrifuge: Eppendorf 5417C
4. Laboratory equipment: volumetric flasks, disposable glass pipets, positive displacement pipets, graduated cylinders, stir bars, stir plates, vortexers, autosampler vials, disposable glass vials, 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

Ground water information:

Ground water used in the study was laboratory well water reconstituted for hardness and was prepared in 1900-L batches by fortifying well water according to the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column to remove any potential organic contaminants. All documentation relating to the preparation, storage and handling is maintained by Smithers Viscient.

Surface water information:

The surface water used for this method validation analysis was collected from the Taunton River (SMV Lot No. 29Jun WatC) in Taunton, Massachusetts. The water was collected from an area of the river with approximately 0-1" of overlying water and was determined to have a pH of 6.10 (YSI model pH100 pH meter) and a dissolved oxygen concentration of 6.36 mg/L (YSI model Pro20 dissolved oxygen meter). All documentation relating to the preparation, storage and handling is maintained by Smithers Viscient.

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 100 mL of acetonitrile with 100 mL of purified reagent water. The solution was mixed using a stir bar and stir plate for five minutes.

A 10:90 purified reagent water: acetonitrile (v:v) liquid reagent was typically prepared by combining 50.0 mL of purified reagent water and 450 mL of acetonitrile. 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 mobile phase 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.

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 solution

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	Sub-stock solution
8303-1C	100	1.00					
Mix-Stk 1	10.0	0.100	10.0		Mix-Stk 2	0.100	High-level recovery samples, and sub-stock solution
Mix-Stk 2	0.100	1.00	10.0		Mix-Stk 3	0.0100	LOQ recovery samples, 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 and TCPSA primary analysis

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 confirmatory analysis

Calibration standards were prepared in 90:10 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 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. Calibration standards for TCPSA were prepared by fortifying with the 0.0100 mg/L mixed test substance sub-stock solution to yield test substance concentrations of 0.0100 µg/L.

2.8.2.1 Matrix-Matched Standards

For triallate analysis in ground and surface waters:

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

[a] Samples were diluted with the final fraction of the Control A-1 (ground water) or Control C-1 (surface water) in 50:50 acetonitrile:purified reagent water (v:v).

[b] Sample ID codes included: -g to denote matrix matched ground water standards and –s to denote matrix matched surface water standards.

For TCPSA primary analysis (on T3 column) in ground and surface waters:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Standard Concentration (µg/L)	Sample ID[c]
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	0.250	0.0100	MM-Std A-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std B-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std C-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std A-s-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std B-s-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std C-s-1

[a] Samples were diluted with the final fraction of the Control A-1 (ground water and surface water) in 50/50 acetonitrile:purified reagent water (v:v).

[b] Dilution solvent: 50:50 acetonitrile:purified reagent water (v:v).

[c] Sample ID codes included: -g to denote matrix matched ground water standards and –s to denote matrix matched surface water standards.

For TCPSA confirmatory analysis (on HILIC column) in ground and surface waters:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume[a] (mL)	Standard Concentration (µg/L)	Sample ID[b]
Mix-Stk 4	0.00100	0.0100	1.00	0.0100	MM-Std G-g
		0.0100	1.00	0.0100	MM-Std H-g
		0.0100	1.00	0.0100	MM-Std I-g
		0.0100	1.00	0.0100	MM-Std G-s
		0.0100	1.00	0.0100	MM-Std H-s
		0.0100	1.00	0.0100	MM-Std I-s

[a] Samples were diluted with the final fraction of the Control A-2 (ground water and surface water) in 90:10 acetonitrile:purified reagent water (v:v).

[b] Sample ID codes included: -g to denote matrix matched ground water standards and –s to denote matrix matched surface water standards.

2.8.2.2 Non Matrix-Matched Standards

For triallate analysis in ground and surface waters:

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

[a] Samples were diluted with 50:50 acetonitrile:purified reagent water (v:v).
[b] Sample ID codes included: -g to denote matrix matched ground water standards and –s to denote matrix matched surface water standards.

For TCPSA primary analysis (on T3 column) in ground and surface waters:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[a] (mL)	Standard Concentration (µg/L)	Sample ID[b]
Mix-Stk 3	0.0100	0.0100	2.00	0.0500	0.250	0.0100	MM-Std A-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std B-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std C-g-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std A-s-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std B-s-1
		0.0100	2.00	0.0500	0.250	0.0100	MM-Std C-s-1

[a] Dilution solvent: 50:50 acetonitrile:purified reagent water (v:v).
[b] Sample ID codes included: -g to denote matrix matched ground water standards and –s to denote matrix matched surface water standards.

For validation with TCPSA confirmatory analysis (on HILIC column) in ground and surface waters:

Test Substance Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume[a] (mL)	Standard Concentration (µg/L)	Sample ID[b]
Mix-Stk 4	0.00100	0.0200	2.00	0.0100	Std G-g
		0.0200	2.00	0.0100	Std H-g
		0.0200	2.00	0.0100	Std I-g
		0.0200	2.00	0.0100	Std G-s
		0.0200	2.00	0.0100	Std H-s
		0.0200	2.00	0.0100	Std I-s

[a] Samples were diluted with 90:10 acetonitrile:purified reagent water (v:v).
[b] Sample ID codes included: -g for ground water and –s for surface water.

2.9 Sample Fortification and Preparation

For each aqueous matrix, a total of twelve recovery samples (5.00 mL sample volume) were transferred into individual disposable glass vials and were fortified with the appropriate test substance mixed sub-stock solution at concentrations of 0.100 and 1.00 µg/L. 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 substance or matrix) in order to assess interference from extraction solvents. The dosing procedure is detailed in the following tables.

Recovery samples in ground water:

Sample ID	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Sample Volume (mL)	Fortified Concentration (µg/L)
Reagent BLK-1	NA[a]	NA	5.00[b]	0.00
Control A & B	NA	NA	5.00	0.00
LOQ A, B, C, D, & E	0.0100	0.0500	5.00	0.100
High A, B, C, D, & E	0.100	0.0500	5.00	1.00

[a] NA = Not Applicable.
[b] The reagent blank sample is purified reagent water.

Recovery samples in surface water:

Sample ID	Sub-Stock Concentration (mg/L)	Fortification Volume (mL)	Sample Volume (mL)	Fortified Concentration (µg/L)
Reagent BLK-2	NA[a]	NA	5.00[b]	0.00
Control C & D	NA	NA	5.00	0.00
LOQ F, G, H, I, & J	0.0100	0.0500	5.00	0.100
High F, G, H, I, & J	0.100	0.0500	5.00	1.00

[a] NA = Not Applicable.
[b] The reagent blank sample is purified reagent water.

2.10 Sample Dilution

To minimize the potential for losses of the test substance during processing, the aqueous test samples were not sub-sampled prior to dilution. The first dilution with acetonitrile was performed by the addition of the reagent to the entire volume of the aqueous sample in the container in which it was fortified. Triallate and TCPSA primary analysis (on T3 column) recovery samples were subsequently diluted into the calibration standard range with 50:50 acetonitrile:purified reagent water (v:v) prior to analysis. TCPSA confirmatory analysis

(on HILIC column) recovery samples were subsequently diluted into the calibration standard range with 90:10 acetonitrile:purified reagent water (v:v) prior to analysis. All samples were centrifuged at 13,000 rpm for 5 minutes prior to analysis in low-binding micro 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 dilution procedures are outlined in the tables below.

2.10.1 Triallate

For triallate primary and confirmatory analysis in ground water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-1-1	0.00	5.00	10.0	NA[c]		2.00
Control A-1 & B-1	0.00	5.00	10.0			2.00
LOQ A-1, B-1, C-1, D-1, & E-1	0.100	5.00	10.0			2.00
High A-1, B-1, C-1, D-1, & E-1	1.00	5.00	10.0	0.500	5.00	20.0

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

For triallate primary and confirmatory analysis in surface water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-2-1	0.00	5.00	10.0	NA[c]		2.00
Control C-1 & D-1	0.00	5.00	10.0			2.00
LOQ F-1, G-1, H-1, I-1, & J-1	0.100	5.00	10.0			2.00
High F-1, G-1, H-1, I-1, & J-1	1.00	5.00	10.0	0.500	5.00	20.0

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

2.10.2 TCPSA

For TCPSA primary analysis (on T3 column) in ground water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-1-1-1	0.00	5.00	10.0	0.250	1.25	10.0
Control A-1-1 & B-1-1	0.00	5.00	10.0	0.250	1.25	10.0
LOQ A-1-1, B-1-1, C-1-1, D-1-1, & E-1-1	0.100	5.00	10.0	0.250	1.25	10.0
High A-1, B-1, C-1, D-1, & E-1	1.00	5.00	10.0	0.500	5.00	20.0

[a] Dilution solvent: acetonitrile.
[b] Dilution solvent: 50:50 acetonitrile:purified reagent water (v:v).

For TCPSA primary analysis (on T3 column) in surface water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-2-1-1	0.00	5.00	10.0	0.250	1.25	10.0
Control C-1-1 & D-1-1	0.00	5.00	10.0	0.250	1.25	10.0
LOQ F-1-1, G-1-1, H-1-1, I-1-1, & J-1-1	0.100	5.00	10.0	0.250	1.25	10.0
High F-1, G-1, H-1, I-1, & J-1	1.00	5.00	10.0	0.500	5.00	20.0

[a] Dilution solvent: acetonitrile.
[b] Dilution solvent: 50:50 acetonitrile:purified reagent water (v:v).

For TCPSA confirmatory analysis (on HILIC column) in ground water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[a] (mL)	Tertiary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-1-2	0.00	5.00	10.0	1.00	5.00	NA[c]		10.0
Control A-2 & B-2	0.00	5.00	10.0	1.00	5.00			10.0
LOQ A-3, B-3, C-2, D-2, & E-2	0.100	5.00	10.0	1.00	5.00			10.0
High A-2, B-2, C-2, D-2, & E-2	1.00	5.00	10.0	NA		0.500	5.00	20.0

[a] Dilution solvent: acetonitrile.
[b] Dilution solvent: 90:10 acetonitrile:purified reagent water (v:v).
[c] NA = Not Applicable.

For TCPSA confirmatory analysis (on HILIC column) in surface water:

Sample ID	Nominal Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[a] (mL)	Tertiary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent BLK-2-2	0.00	5.00	10.0	1.00	5.00	NA[c]		10.0
Control C-2 & D-2	0.00	5.00	10.0	1.00	5.00			10.0
LOQ F-2, G-2, H-2, I-2, & J-2	0.100	5.00	10.0	1.00	5.00			10.0
High F-2, G-2, H-2, I-2, & J-2	1.00	5.00	10.0	NA		0.500	5.00	20.0

[a] Dilution solvent: acetonitrile.
[b] Dilution solvent: 90:10 acetonitrile:purified reagent water (v:v).
[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
Ground water	Primary	3.88	304.1 / 86.1	24.70
	Confirmatory	3.87	304.1 / 142.8	41.00
Surface water	Primary	3.87	304.1 / 86.1	24.70
	Confirmatory	3.86	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 (on T3 column) Parameters:

Column:	Atlantis® T3, 3 µm, 4.6 × 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	1.20	50.0	50.0
3.00	1.20	50.0	50.0

Run Time:	3.0 minutes
Injector Rinse Solvent 1:	30:30:40 acetonitrile:methanol:purified reagent water (v:v:v)
Column Temperature:	40 °C
Sample Temperature:	5 °C
Injection Volume:	100 µL

LC (on HILIC column) 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	0.300	10.0	90.0
5.00	0.300	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:	100 µL

MS Parameters (both primary T3 and confirmatory HILIC column analyses):

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
Ground water	Primary (T3 Column)	1.63	224.8 / 79.8	-50.00
	Confirmatory (HILIC column)	2.03	224.8 / 79.8	-50.00
Surface water	Primary (T3 Column)	1.66	224.8 / 79.8	-50.00
	Confirmatory (HILIC column)	2.02	224.8 / 79.8	-50.00

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 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 (0.100 µg/L). 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 1.4.2 or 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/L), 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.

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

where:

MDL_{LCAL}	=	the lowest concentration calibration standard (0.00500 µg/L)
DF_{CNTL}	=	dilution factor of the control samples (smallest dilution factor used: 2.00 for triallate and 10.0 for TCSPA)
MDL	=	method detection limit reported for the analysis of triallate, or TCPSA recovery samples (0.0100 µg/L triallate and 0.0500 µg/L TCPSA)