SUMMARY – Part A

Methodology was validated (April 2013) to quantify the amount of TCVP and TCBA present in soil. Recovery samples containing both TCVP and TCBA were extracted a total of two times with 80:20 acetonitrile:purified reagent water (v:v). The extracts were combined and brought to a total volume of 50.0 mL with 80:20 acetonitrile:purified reagent water (v:v). All recovery samples were further diluted with purified reagent water, while the high-level recovery samples were additionally diluted into the calibration curve with 20:80 acetonitrile:purified reagent water (v:v). All samples were analyzed by liquid chromatography with mass spectrometry detection (LC/MS/MS). An attempt to quantify TCCEol, TCPEol and TCPEone was also performed using the same extraction procedure; however this attempt was unsuccessful. Therefore, TCCEol TCPEol and TCPEone were quantified separately following a different extraction procedure and the method is presented in Part B.

EXPERIMENTAL

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 AG 285, Mettler PJ 3000, Sartorius Moisture Analyzer MA-45
3. Centrifuge:	Beckman Allegra X-12, Eppendorf 5417C
4. Shaker table:	Labline Orbital Shaker Table
5. Laboratory equipment:	volumetric flasks, disposable glass pipets, positive displacement pipets, disposable glass vials, Nalgene centrifuge tubes, autosampler vials, and amber glass bottles with Teflon®-lined caps

Reagents

1. Acetonitrile:	EMD and Burdick & Jackson, reagent grade
2. Ammonium acetate:	EMD, reagent grade
3. Purified reagent water:	Prepared from a Millipore MilliQ® Direct 8 water purification system (meets ASTM Type II requirements)

Test Substances

The test substance, tetrachlorvinphos (TCVP), was received on 12 August 2011 from The Hartz Mountain Corporation, Bloomfield, New Jersey. The following information was provided:

Name:	tetrachlorvinphos (TCVP)
Synonym:	"Rabon" Insecticide, Technical
CAS No.:	22248-79-9
Lot No.:	TX 100113
Purity:	99.51%
Expiration Date:	19 March 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5193) was stored at room temperature in the original container in a dark, ventilated cabinet.

The test substance, 2,4,5-trichlorobenzoic acid (TCBA), was received on 15 May 2012 from Chemtos, Austin, Texas. The following information was provided:

Name:	2,4,5-trichlorobenzoic acid (TCBA)
Lot No.:	C5-124-031
CAS No.:	Not Listed
Purity:	99.9%
Retest Date:	31 May 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5629) was stored frozen in the original container.

Concentrations were adjusted for the purity of each of the test substances.

PROCEDURES

Standard Reagents

All aqueous solutions were prepared using purified reagent water (meeting ASTM Type II requirements) obtained with a Millipore MilliQ® Direct 8 purification system. The filter-sterilized water typically has greater than 16.7 MΩ-cm resistivity and less than 1 mg/L total organic carbon, which is the established limit at this laboratory. All chemicals and solvents were at least reagent grade and were obtained from commercial sources.

Preparation of Standard Reagents and Mobile Phase

An 80:20 acetonitrile:purified reagent water liquid reagent was typically prepared by combining 200 mL of purified reagent water with 800 mL of acetonitrile and mixed using a stir bar and stir plate for 10 minutes.

A 20:80 acetonitrile:purified reagent water liquid reagent was prepared by combining 400 mL of purified reagent water with 100 mL of acetonitrile and mixed using a stir bar and stir plate for 10 minutes.

A 10 mM ammonium acetate in purified reagent water mobile phase was prepared by adding approximately 0.7704 g of ammonium acetate to 1000 mL of purified reagent water. This mobile phase was mixed well and degassed under vacuum with sonication.

Test Soil

The soil used for the method validation was Mutchler Sandy Loam (MSL) soil (SMV Lot No. 122812BS, Sample ID MSL-PF 4 to 8") from Grand Forks, North Dakota, USA which was collected by Agvise Laboratories personnel. The soils were stored refrigerated in the dark until needed for analysis. Prior to testing, soil moisture content of the MSL soil was 12.65% using a Sartorius MA-45 moisture analyzer.

Preparation of Stock Solutions

Primary stock solutions were typically prepared in acetonitrile from each of the test substances as outlined in the table below.

Test Material	Amount of Substance Weighed (g), Net Weight	Amount of Substance Weighed (g), as Active Ingredient	Final Volume (mL)	Primary Stock Concentration (mg/mL)	Primary Stock Use
TCVP	0.01013	0.01008	10.0	1.01	To prepare secondary stock solutions
TCBA	0.01010	0.01009	10.0	1.01	To prepare secondary stock solutions

Secondary stock solutions were typically prepared in acetonitrile from the appropriate 1.01 mg/mL primary stock solutions as outlined in the table below.

Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Diluted to a Final Volume (mL)	Stock Concentration (mg/L)	Stock Use
1010 (TCVP)	0.250	25.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	25.0	101	To prepare sub-stock solution for recovery samples and calibration standards
1010 (TCBA)	0.250	25.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	25.0	101	To prepare sub-stock solution for recovery samples and calibration standards

Mixed stock solutions were prepared in acetonitrile by combining the appropriate TCVP and TCBA stock solutions as summarized in the following table.

Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Concentration (mg/L)	Stock ID	Stock Use
10.1 (TCVP)	0.100	10.0	0.101	Mix Stk 1	Low-level recovery samples and calibration standards
10.1 (TCBA)	0.100				
101 (TCVP)	0.100	10.0	1.01	Mix Stk 2	High-level recovery samples and sub-stock for calibration standards
101 (TCBA)	0.100				
Mix Stk 2	0.100	10.0	0.0101	Mix Stk 3	Calibration standards

All primary and secondary stock solutions were stored in a refrigerator in amber glass bottles fitted with Teflon®-lined caps. All mixed stock solutions were prepared on the day of use and discarded after use.

Preparation of Calibration Standards

Calibration standards were prepared in 20:80 acetonitrile:purified reagent water (v:v) by fortifying with the appropriate mix stock solution as described in the following table.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)
Mix Stk 3	0.0101	0.0350	10.0	0.0354
Mix Stk 3	0.0101	0.0500	10.0	0.0505
Mix Stk 3	0.0101	0.100	10.0	0.101
Mix Stk 1	0.101	0.0250	10.0	0.253
Mix Stk 1	0.101	0.0500	10.0	0.505
Mix Stk 1	0.101	0.100	10.0	1.01
Mix Stk 1	0.101	0.250	10.0	2.53

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. Five samples of each concentration were dosed with the appropriate mixed sub-stock solution at 10.1 and 101 µg/kg (dry weight). Two samples were left untreated and were designated as controls. The dosing procedure is detailed in the following table:

Sample ID	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Dry weight (g)	Nominal Concentration (µg/kg)
Control A & B	NA	NA	NA	5.00	0.00
Low A, B, C, D & E	Mix Stk 1	0.101	0.500	5.00	10.1
High A, B, C, D & E	Mix Stk 2	1.01	0.500	5.00	101

Sediment Extraction and Dilution

A 20.0-mL aliquot of 80:20 acetonitrile:purified reagent water (v:v) 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 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 80:20 acetonitrile:purified reagent water (v:v). The second extract was combined with the first in the appropriate volumetric flasks and taken to a final volume of 50.0 mL with 80:20 acetonitrile:purified reagent water (v:v). Samples were further diluted into the standard calibration range with purified reagent water, with high-level samples being additionally diluted with 20:80 acetonitrile:purified reagent water (v:v). The extraction and dilution procedures are detailed below.

Sample ID	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[c] (mL)	Dilution Factor
Control A & B	0.00	5.00	20.0	50.0	1.25	5.00	NA[d]		40.0
Low A, B C, D& E	10.1	5.00	20.0	50.0	1.25	5.00	NA		40.0
High A, B C, D& E	101	5.00	20.0	50.0	1.25	5.00	1.00	10.0	400

[a] Extracted and diluted with 80:20 acetonitrile:purified reagent water (v:v).
[b] Dilution solvent: purified reagent water
[c] Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v).
[d] NA=Not Applicable

ANALYSIS

Instrumental Conditions

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

LC parameters:

Column: Acquity BEH C18 1.7µm, 2.1 x 50 mm
Mobile Phase A: 10 mM Ammonium acetate in purified reagent water
Mobile Phase B: Acetonitrile
Gradient:

Time (min.)	Flow rate (mL/min)	Solvent A	Solvent B
0.00	0.600	95.0%	5.0%
0.50	0.600	95.0%	5.0%
3.00	0.600	5.0%	95.0%
4.00	0.600	5.0%	95.0%
4.10	0.600	95.0%	5.0%
5.00	0.600	95.0%	5.0%

Injection volume: 100 µL
Column oven: 40 °C
Sample temperature: 5 °C
Retention Time: Approximately 2.6 minutes (for TCVP)
Approximately 1.5 minutes (for TCBA)

MS parameters:

Instrument: AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source
Q1/Q3 Mass: 366.313/126.900 Da (for TCVP)
223.000/179.000 Da (for TCBA)
Dwell Time: 500 milliseconds
Source temperature: 400 °C
Scan type: MRM

Instrument Parameter:	Experiment 1: TCBA (0 - 2 minutes)	Experiment 2: TCVA (2 - 5 minutes)
Ionization Mode	Negative	Positive
Resolution Q1/Q3	Low/Unit	Low/Unit
Curtain Gas	45.00	45.00
Ion Source Gas 1/Gas 2	75.00/70.00	15.00/30.00
Ion Spray Voltage	-4500.00	3000.00
Collision Gas	4.50	4.50
Declustering Potential	-35.00	111.00
Entrance Potential	-10.00	10.00
Collision Energy	-14.00	25.00
Collision Cell Exit Potential	-17.00	48.00

Preparation of 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 recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

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.

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

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

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

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 volume)
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

SUMMARY - Part B

Methodology was validated (June 2013) to quantify the amount of TCCEol, TCPEol and TCPEone present in soil. Recovery samples containing a mixture of the test substances were extracted a total of two times with acetonitrile. The extracts were combined and brought to a total volume of 50.0 mL with acetonitrile. Due to the lower sensitivity of TCCEol, a portion of the extract was taken to dryness and reconstituted with 20:80 acetonitrile:purified reagent water (v:v) for the analysis of TCCEol. All TCCEol recovery samples were further diluted into the calibration curve with 20:80 acetonitrile:purified reagent water (v:v). Samples analyzed for TCPEol and TCPEone were diluted with 20:80 acetonitrile purified reagent water without nitrogen concentration. All samples were analyzed by liquid chromatography with mass spectrometry detection (LC/MS/MS).

EXPERIMENTAL

Equipment

See Part A

Reagents

1. Acetonitrile:	EMD, reagent grade
2. Ammonium carbonate:	Sigma Aldrich, reagent grade
3. Purified reagent water:	Prepared from a Millipore MilliQ® Direct 8 water purification system (meets ASTM Type II requirements)

Test Substances

The test substance, 2,4,5-trichloroacetophenone (TCPEone), was received on 30 May 2012 from Overton and Company, Elmont, New York. The following information was provided:

Name:	2,4,5-trichloroacetophenone (TCPEone)
Lot No.:	1492A-4-2A
CAS No.:	22248-79-9
Purity:	99.9%
Re-Test Date:	3 June 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5641) was stored frozen in the original container.

The test substance, 1-(2,4,5-trichlorophenyl)-ethanol (TCPEol), was received on 30 May 2012 from Overton and Company, Elmont, New York. The following information was provided:

Name:	1-(2,4,5-trichlorophenyl)-ethanol (TCPEol)
Lot No.:	1492A-5-1A
CAS No.:	22248-79-9
Purity:	99.9%
Retest Date:	31 May 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5642) was stored frozen in the original container.

The test substance, 1-(2,4,5-trichlorophenyl)-2-chloroethanol (TCCEol), was received on 15 May 2012 from Chemtos, Austin, Texas. The following information was provided:

Name:	1-(2,4,5-trichlorophenyl)-2-chloroethanol (TCCEol)
Lot No.:	C2-117-150
CAS No.:	Not Listed
Purity:	99.9%
Retest Date:	9 May 2013

Upon receipt at Smithers Viscient, the test substance (SMV No. 5628) was stored frozen in the original container.

Concentrations were adjusted for the purity of each of the test substances.

PROCEDURES

Standard Reagents

See Part A

Preparation of Standard Reagents and Mobile Phase

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

A 10 mM ammonium carbonate in purified reagent water mobile phase was prepared by adding approximately 1.92 g of ammonium carbonate to 2000 mL of purified reagent water. This mobile phase was well mixed and degassed under vacuum with sonication.

Test Soil

The soil used for the method validation was Mutchler Sandy Loam (MSL) soil (SMV Lot No. 122812BS, Sample ID MSL-PF 4 to 8") from Grand Forks, North Dakota, USA which was collected by Agvise Laboratories personnel. The soils were stored refrigerated in the

dark until needed for analysis. Prior to testing, soil moisture content of the MSL soil was 13.87% using a Sartorius MA-45 moisture analyzer.

Preparation of Stock Solutions

Primary stock solutions were typically prepared in acetonitrile from each of the test substances as outlined in the table below.

Test Material	Amount of Substance Weighed (g), Net Weight	Amount of Substance Weighed (g), as Active Ingredient	Final Volume (mL)	Primary Stock Concentration (mg/mL)	Primary Stock Use
TCPEone	0.01005	0.01004	10.0	1.00	To prepare secondary stock solutions
TCPEol	0.01006	0.01005	10.0	1.00	To prepare secondary stock solutions
TCCEol	0.01006	0.01005	10.0	1.00	To prepare secondary stock solutions

Secondary stock solutions were typically prepared in acetonitrile from the appropriate 1.00 mg/mL primary stock solutions as outlined in the table below.

Fortifying Stock Concentration (mg/mL)	Volume of Fortification (mL)	Diluted to a Final Volume (mL)	Stock Concentration (mg/L)	Stock Use
1.00 (TCPEone)	0.250	25.0	10.0	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	25.0	100	To prepare sub-stock solution for recovery samples and calibration standards
1.00 (TCPEol)	0.250	25.0	10.0	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	25.0	100	To prepare sub-stock solution for recovery samples and calibration standards
1.00 (TCCEol)	0.250	25.0	10.0	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	25.0	100	To prepare sub-stock solution for recovery samples and calibration standards

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

Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Concentration (mg/L)	Stock ID	Stock Use
10.0	0.100	10.0	0.100	TCCEol Stk 1	Low-level recovery samples and calibration standards
100	0.100	10.0	1.00	TCCEol Stk 2	High-level recovery samples and calibration standards

Mixed stock solutions were prepared in acetonitrile by combining TCPEone and TCPEol as summarized in the following table.

Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Concentration (mg/L)	Stock ID	Stock Use
10.0 (TCPEone)	0.100	10.0	0.100	Mix Stk 1	Low-level recovery samples and calibration standards
10.0 (TCPEol)	0.100				
100 (TCPEone)	0.100	10.0	1.00	Mix Stk 2	High-level recovery samples and calibration standards
100 (TCPEol)	0.100				
Mix Stk 2	0.100	10.0	0.0100	Mix Stk 3	Calibration standards

All primary and secondary stock solutions were stored in a refrigerator in amber glass bottles fitted with Teflon®-lined caps. All mixed stock and sub-stock solutions were prepared on the day of use and discarded after use.

Preparation of Calibration Standards

Calibration standards were prepared in 20:80 acetonitrile:purified reagent water (v:v) by fortifying with the appropriate mix stock and sub-stock solutions as described in the following table.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)	Sample ID
Mix Stk 3	0.0100	0.0350	10.0	0.0350	Std 1
TCCEol Stk 1	0.100	0.0500	10.0	0.500	
Mix Stk 3	0.0100	0.0500	10.0	0.0500	Std 2
TCCEol Stk 1	0.100	0.0750	10.0	0.750	
Mix Stk 3	0.0100	0.100	10.0	0.100	Std 3
TCCEol Stk 1	0.100	0.100	10.0	1.00	
Mix Stk 1	0.100	0.0250	10.0	0.250	Std 4
TCCEol Stk 1	0.100	0.250	10.0	2.50	
Mix Stk 1	0.100	0.0500	10.0	0.500	Std 5
TCCEol Stk 2	1.00	0.0500	10.0	5.00	
Mix Stk 1	0.100	0.100	10.0	1.00	Std 6
TCCEol Stk 2	1.00	0.100	10.0	10.0	
Mix Stk 2	1.00	0.0250	10.0	2.50	Std 7
TCCEol Stk 2	1.00	0.250	10.0	25.0	

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. Five samples of each concentration were dosed with the appropriate test substance mixed or sub-stock solutions at 10.0 and 100 µg/kg (dry weight). Two samples were left untreated and were designated as controls. The dosing procedure is detailed in the following table:

Sample ID	Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Dry weight (g)	Nominal Concentration (µg/kg)
Control A & B	NA	NA	NA	5.00	0.00
Low A, B, C, D & E	Mix Stk 1 & TCCEol Stk 1	0.100	0.500	5.00	10.0
High A, B, C, D & E	Mix Stk 2 & TCCEol Stk 2	1.00	0.500	5.00	100

Sediment Extraction and Dilution

A 20.0-mL aliquot of 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 for 10 minutes and the extracts were transferred to labeled 50.0-mL

volumetric flasks. The extraction and centrifugation procedure was repeated with 20.0-mL aliquots of acetonitrile. The second extract was combined with the first in the appropriate volumetric flasks and taken to 50.0-mL final volume with acetonitrile and mixed well. A sample of the extracts was stored refrigerated in amber Wheaton bottles for future analysis, if needed. Due to the lower sensitivity of TCCEol, a portion of the combined extract was transferred to a graduated glass conical and taken to dryness under a gentle stream of nitrogen at room temperature (these samples were denoted "-1"). The concentrated samples were then reconstituted with 20:80 acetonitrile:purified reagent water (v:v). The extraction and dilution procedure for the analysis of TCCEol are presented in the table below.

Sample ID (-1)	Nominal Concentration (µg/kg)	Dry weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Control A & B	0.00	5.00	20.0	50.0	10.0	2.00	2.00
Low A, B C, D& E	10.0	5.00	20.0	50.0	10.0	2.00	2.00
High A, B C, D& E	100	5.00	20.0	50.0	2.50	5.00	20.0

[a] Extracted and diluted with acetonitrile.
[b] *Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v).*

The initial analysis for TCPEol and TCPEone was not successful when following the same procedure as the TCCEol samples (i.e., taken to dryness under a gentle stream of nitrogen at room temperature). It was determined that the concentration step resulted in recovery losses for TCPEol and TCPEone. Therefore, the original acetonitrile extracts were removed from the refrigerator mixed well for the analysis of TCPEol and TCPEone (these samples were denoted "-3"). Each sample was then further diluted with 20:80 acetonitrile:purified reagent water, without nitrogen concentration. Each sample was centrifuged at 13,000 rpm for 5 minutes to remove any particulates. The extraction and dilution procedure for TCPEol and TCPEone is presented in the table below.

Sample ID (-3)	Nominal Concentration (µg/kg)	Dry weight (g)	Extract Volume[a] (mL)	Final Volume (mL)[a]	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Control A & B	0.00	5.00	20.0	50.0	0.500	5.00	100
Low A, B C, D& E	10.0	5.00	20.0	50.0	0.500	5.00	100
High A, B C, D& E	100	5.00	20.0	50.0	0.500	50.0	1000

[a] Extracted and diluted with acetonitrile; stored refrigerated prior to dilution.
[b] Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v).

ANALYSIS

Instrumental Conditions

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

LC parameters:

Column: Acquity BEH C18 1.7µm, 2.1 x 150 mm
Mobile Phase A: 10 mM Ammonium carbonate in purified reagent water
Mobile Phase B: Acetonitrile
Gradient:

Time (min.)	Flow rate (mL/min)	Solvent A	Solvent B
0.00	0.300	80.0%	20.0%
0.50	0.300	80.0%	20.0%
3.50	0.300	45.0%	55.0%
12.00	0.300	45.0%	55.0%
12.10	0.300	80.0%	20.0%
15.00	0.300	80.0%	20.0%

Injection volume: 100 µL
Column oven: 65 °C
Sample temperature: 5 °C
Retention Time: Approximately 6.4 minutes (for TCCEol)
Approximately 6.1 minutes (for TCPEol)
Approximately 6.7 minutes (for TCPEone)

MS parameters:

Instrument: AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V APCI Ion Spray source
Ionization Mode: Negative
Q1/Q3 Mass: 202.800/160.970 Da
Dwell Time: 500 milliseconds
Source temperature: 500 °C
Scan type: MRM

Instrument Parameter:	*Experiment 1: TCCEol, TCPEol, TCPEone*
Ionization Mode	Negative
Resolution Q1/Q3	Low/Unit
Curtain Gas	40.00
Ion Source Gas 1/Gas 2	55.00/0.00
Nebulizer Current	-3.00
Collision Gas	4.50
Declustering Potential	-35.00
Entrance Potential	-10.00
Collision Energy	-25.00
Collision Cell Exit Potential	-15.00

Preparation of 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 recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

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) $y = ax^2 + bx + c$

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

(3) $A = DC \times DF$

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 volume)
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