1.0 INTRODUCTION

Methodology from Smithers Viscient (SMV No. 14020.6109) was validated (5 and 7 November 2013) to quantify the concentration of TCVP, TCBA, TCCEol, TCPEol and TCPEone present in recovery samples prepared in soil from 5 to 7 November 2013. The detailed method is presented in Appendix 1 of the protocol (Appendix 1, Smith, 2014). This independent laboratory validation (ILV) study is required by U.S. EPA under Guideline No. 850.6100 (U.S. EPA, 2012) to confirm that the original analytical method, developed by one group, can be independently validated by a second group with no major interaction between the two groups. This method was validated by fortification of soil with TCVP, TCBA, TCCEol, TCPEol and TCPEone at concentrations of approximately 10.0 µg/kg (LOQ) and 100 µg/kg (10X LOQ). 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). 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). Recovery samples containing a mixture of TCCEol, TCPEol and TCPEone 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 recovery samples were then analyzed using liquid chromatography with mass spectrometry (LC/MS/MS).

The study was initiated on 1 November 2013, 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 ILV study was conducted on 5 to 7 November 2013 at Smithers Viscient (SMV), located in Wareham, Massachusetts. A final report for this study was issued to TCVP Task Force dated

28 February 2014. This amended final report, 5 March 2014, incorporates changes made as presented in the Final Report Amendment, which is appended as the last page of this report. 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 Study Protocol

This study was performed following the Smithers Viscient protocol entitled "Independent Laboratory Validation (ILV) of the Analytical Method: Method Validation for TCVP, TCBA, TCCEol, TCPEol and TCPEone in Soil by Liquid Chromatography with Mass Spectrometry Detection (14020.6109)", (Appendix 1). The methods described in this protocol meet the requirements specified in the OCSPP Guideline 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (U.S. EPA, 2012) and OSCPP Guideline 850.7100: Data Reporting for Environmental Chemistry Methods (U.S. EPA, 1996).

2.2 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. Concentrations were adjusted for the purity of this test substance.

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 the test substance.

The test substance, 2,4,5-trichloroacetophenone (TCPEone), was received on 4 October 2011 from Paragon Global Services, Suffolk, United Kingdom. The following information was provided:

Name:	2,4,5-trichloroacetophenone (TCPEone)
CAS No.:	Not Listed
Lot No.:	1492A-4-2A
Purity:	99.9%
Retest Date:	3 June 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5258) was stored frozen in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, 1-(2,4,5-trichlorophenyl)-ethanol (TCPEol), was received on 4 October 2011 from Paragon Global Services, Suffolk, United Kingdom. The following information was provided:

Name:	1-(2,4,5-trichlorophenyl)-ethanol (TCPEol)
CAS No.:	Not Listed
Lot No.:	1492A-5-1A
Purity:	99.9%
Retest Date:	31 May 2014

Upon receipt at Smithers Viscient, the test substance (SMV No. 5261) was stored frozen in the original container. Concentrations were adjusted for the purity of the test substance.

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:	3 June 2014

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 the test substance.

The test substances were used to fortify the recovery samples and prepare the calibration standards during testing. 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.	Ammonium carbonate:	Sigma Aldrich, reagent grade
3.	Purified reagent water:	prepared from a Millipore Milli-Q® Direct 8 system (meeting ASTM Type II requirements)
4.	Ammonium acetate:	BDH, reagent grade

2.4 Equipment

1.	Instrument:	AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source, an Acquity Sample Manager autosampler, an Acquity Binary Solvent Manager binary pump, an Acquity Column Compartment column oven, and Analyst 1.6 software for data acquisition
2.	Balance:	Mettler PJ-3000, Mettler Toledo AG 240, Mettler Toledo AG 285, Sartorius Moisture Analyzer MA-45
3.	Centrifuge:	Beckman Allegra X-12, Eppendorf 5417C
4.	Shaker table:	Orbit Shaker Table 3520
5.	Laboratory equipment:	volumetric flasks, disposable glass pipets, disposable glass vials, positive displacement pipets, Nalgene centrifuge tubes, autosampler vials, and amber glass bottles with Teflon®-lined caps

2.5 Test Soil

The soil used for this ILV analysis was Mutchler Sandy Loam (MSL) soil (SMV Lot No. 10091313, Sample ID MSL-PF 0 to 6") from Grand Forks, North Dakota, USA which was collected by Agvise Laboratories personnel. The soil was stored refrigerated in the dark until needed for analysis. Prior to testing, soil moisture content of the MSL soil was 18.96% for the TCVP and TCBA analysis, 17.94% for the TCPEol and TCPEone analysis and 21.13% for the TCCEol analysis using a Sartorius MA-45 moisture analyzer.

2.6 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/L)	Primary Stock Use
TCVP	0.01009	0.01004	10.0	1000	To prepare secondary stock solutions
TCBA	0.01006	0.01005	5.00	2010	To prepare secondary stock solutions
TCPEone	0.01006	0.01005	5.00	2010	To prepare secondary stock solutions
TCPEol	0.01005	0.01004	5.00	2010	To prepare secondary stock solutions
TCCEol	0.01004	0.01003	5.00	2010	To prepare secondary stock solutions

Secondary stock solutions were typically prepared in acetonitrile from the appropriate primary stock solution 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
1000 (TCVP)	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
2010 (TCBA)	0.250	50.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	50.0	101	To prepare sub-stock solution for recovery samples and calibration standards
2010 (TCPEone)	0.250	50.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	50.0	101	To prepare sub-stock solution for recovery samples and calibration standards
2010 (TCPEol)	0.250	50.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	50.0	101	To prepare sub-stock solution for recovery samples and calibration standards
2010 (TCCEol)	0.250	50.0	10.1	To prepare sub-stock solution for recovery samples and calibration standards
	2.50	50.0	101	To prepare sub-stock solution for recovery samples and calibration standards

Mixed stock solutions that combined TCVP and TCBA stock solutions were prepared in acetonitrile as summarized in the following table.

Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Concentration (mg/L)[a]	Stock ID	Stock Use
10.0 (TCVP)	0.100	10.0	0.100/0.101	Mix Stk[b] 1	Low-level recovery samples and calibration standards
10.1 (TCBA)	0.100				
100 (TCVP)	0.100	10.0	1.00/1.01	Mix Stk 2	High-level recovery samples and sub-stock for calibration standards
101 (TCBA)	0.100				
Mix Stk 2	0.100	10.0	0.0100/0.0101	Mix Stk 3	Calibration standards

[a] The concentrations are presented as TCVP/TCBA
[b] Stk = Stock.

Mixed stock solutions the combined TCPEone and TCPEol were prepared in acetonitrile 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 (TCPEone)	0.100	10.0	0.101	Mix Stk[a] 1	Low-level recovery samples and calibration standards
10.1 (TCPEol)	0.100				
101 (TCPEone)	0.100	10.0	1.01	Mix Stk 2	High-level recovery samples and calibration standards
101 (TCPEol)	0.100				
Mix Stk 2	0.100	10.0	0.0101	Mix Stk 3	Calibration standards

[a] Stk = Stock.

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
10.1	0.100	10.0	0.101	TCCEol Stk[a] 1	Low-level recovery samples and calibration standards
101	0.100	10.0	1.01	TCCEol Stk 2	High-level recovery samples and calibration standards

[a] Stk = Stock.

All primary and secondary stock solutions were stored refrigerated in glass amber bottles fitted with Teflon®-lined caps. All sub-stock and mixed-stock solutions were prepared daily and discarded after use.

2.7 Reagent Solution and Mobile Phase Preparation

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 solution was prepared by adding approximately 0.7708 g of ammonium acetate to 1000 mL of purified reagent water. This mobile phase was mixed well and degassed under vacuum with sonication.

A 10 mM ammonium carbonate in purified reagent water mobile phase solution was prepared by adding approximately 0.9610 g of ammonium carbonate to 1000 mL of purified reagent water. This mobile phase was well mixed and degassed under vacuum with sonication.

2.8 Preparation of Calibration Standards

2.8.1 TCVP and TCBA

Calibration standards for the TCVP and TCBA analysis 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[a] (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration[a] (µg/L)
Mix Stk[b] 3	0.0100/0.0101	0.0350	10.0	0.0350/0.0354
Mix Stk 3	0.0100/0.0101	0.0500	10.0	0.0500/0.0505
Mix Stk 3	0.0100/0.0101	0.100	10.0	0.100/0.101
Mix Stk 1	0.100/0.101	0.0250	10.0	0.250/0.253
Mix Stk 1	0.100/0.101	0.0500	10.0	0.500/0.505
Mix Stk 1	0.100/0.101	0.100	10.0	1.00/1.01
Mix Stk 1	0.100/0.101	0.250	10.0	2.50/2.53

[a] The concentrations are presented as TCVP/TCBA
[b] Stk = Stock.

2.8.2 TCPEone, TCPEol and TCCEol

Calibration standards for the TCPEone, TCPEol and TCCEol analysis were also 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[a] 3	0.0101	0.0350	10.0	0.0354	Std 1
TCCEol Stk 1	0.101	0.0500	10.0	0.505	
Mix Stk 3	0.0101	0.0500	10.0	0.0505	Std 2
TCCEol Stk 1	0.101	0.0750	10.0	0.758	
Mix Stk 3	0.0101	0.100	10.0	0.101	Std 3
TCCEol Stk 1	0.101	0.100	10.0	1.01	
Mix Stk 1	0.101	0.0250	10.0	0.253	Std 4
TCCEol Stk 1	0.101	0.250	10.0	2.53	
Mix Stk 1	0.101	0.0500	10.0	0.505	Std 5
TCCEol Stk 2	1.01	0.0500	10.0	5.05	
Mix Stk 1	0.101	0.100	10.0	1.01	Std 6
TCCEol Stk 2	1.01	0.100	10.0	10.1	
Mix Stk 2	1.01	0.0250	10.0	2.53	Std 7
TCCEol Stk 2	1.01	0.250	10.0	25.3	

[a] Stk = Stock.

2.9 Sample Fortification and Preparation

All soil recovery samples (5.00 g dry weight) were weighed into individual 50.0-mL Nalgene® centrifuge tubes. Five replicates of each concentration were dosed with the appropriate test substance mixed or sub-stock solutions at 10.0/10.1 and 100/101 µg/kg (dry weight). The dosing procedure is detailed in the following tables:

TCPA and TCBA Analysis:

Sample ID	Stock ID	Fortifying Stock Concentration[a] (mg/L)	Fortification Volume (mL)	Dry weight (g)	Nominal Concentration[a] (µg/kg)
Control A & B	NA[b]	NA	NA	5.00	0.00
Low A, B, C, D & E	Mix Stk[c] 1	0.100/0.101	0.500	5.00	10.0/10.1
High A, B, C, D & E	Mix Stk 2	1.00/1.01	0.500	5.00	100/101

[a] The concentrations are presented as TCVP/TCBA
[b] NA = Not Applicable
[c] Stk = Stock

TCPEone and TCPEol Analysis:

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

[a] NA = Not Applicable
[b] Stk = Stock

2.10 Soil Extraction and Dilution

2.10.1 TCVP and TCBA

A 20.0-mL aliquot of 80:20 acetonitrile:purified reagent water (v:v) was added to the soil recovery samples (5.00 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[a] (µg/kg)	Dry weight (g)	Extract Volume[b] (mL)	Final Volume[b] (mL)	Secondary Volume (mL)	Final Volume[c] (mL)	Tertiary Volume (mL)	Final Volume[d] (mL)	Dilution Factor
Control A & B	0.00	5.00	20.0	50.0	1.25	5.00	NA[e]		40.0
Low A, B, C, D & E	10.0/10.1	5.00	20.0	50.0	1.25	5.00	NA		40.0
High A, B, C, D & E	100/101	5.00	20.0	50.0	1.25	5.00	1.00	10.0	400

[a] Concentrations are presented as TCVP/TCBA
[b] Extracted and diluted with 80:20 acetonitrile:purified reagent water (v:v)
[c] Dilution solvent: purified reagent water
[d] Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v)
[e] NA=Not Applicable

2.10.2 TCPEone, TCPEol and TCCEol

A 20.0-mL aliquot of acetonitrile was added to the soil recovery samples (5.00 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. For the TCPEone and TCPEol analysis (samples were denoted as "-1"), recovery samples were further diluted with 20:80 acetonitrile:purified reagent water and then centrifuged at 13,000 rpm for five minutes. The extraction and dilution procedure for TCPEone and TCCEol analysis are presented in the table below:

Sample ID (-1)	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	10.1	5.00	20.0	50.0	0.500	5.00	100
High A, B C, D & E	101	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).

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 "-2"). The concentrated samples were then reconstituted with 20:80 acetonitrile:purified reagent water (v:v). Samples were then centrifuged at 13,000 rpm for five minutes. The extraction and dilution procedure for the analysis of TCCEol are presented in the table below.

Sample ID (-2)	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	10.1	5.00	20.0	50.0	10.0	2.00	2.00
High A, B C, D & E	101	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).

2.11 Analysis

2.11.1 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 (for TCVP and TCVBA)
10 mM Ammonium carbonate in purified reagent water (for TCPEone, TCPEol and TCCEol)

Mobile Phase B: Acetonitrile

Gradient:

TCVP and TCBA:

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%

TCPEone, TCPEol and TCCEol:

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: 40 °C (TCVP and TCBA)
65 °C (TCPEone, TCPEol and TCCEol)

Sample temperature: 5 °C

Retention Time: Approximately 2.4 minutes (for TCVP)
Approximately 1.3 minutes (for TCBA)
Approximately 6.5 minutes (for TCPEone)
Approximately 6.0 minutes (for TCPEol)
Approximately 6.3 minutes (for TCCEol)

MS parameters:

Instrument: AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI/APCI Ion Spray source

Q1/Q3 Mass: 366.313/126.900 Da (for TCVP)
223.000/179.000 Da (for TCBA)
202.800/160.970 Da (for TCPEone, TCPEol and TCCEol)

Dwell Time: 500 milliseconds

Scan type: MRM

Instrument Parameter:	Experiment 1: TCBA (0 - 2 minutes)	Experiment 2: TCVP (2 - 5 minutes)	Experiment 3: TCPEone, TCPEol and TCCEol (2 – 15 minutes)
Ion Source	ESI	ESI	APCI
Source Temperature (°C)	400	400	500
Ionization Mode	Negative	Positive	Negative
Resolution Q1/Q3	Low/Unit	Low/Unit	Low/Unit
Curtain Gas	45.00	45.00	40.00
Ion Source Gas 1/Gas 2	75.00/70.00	15.00/30.00	55.00/0.00
Ion Spray Voltage	-4500.00	3000.00	NA
Collision Gas	4.50	4.50	4.50
Declustering Potential	-35.00	111.00	-35.00
Entrance Potential	-10.00	10.00	-10.00
Collision Energy	-14.00	25.00	-25.00
Collision Cell Exit Potential	-17.00	48.00	-15.00

NA = Not Applicable.

2.11.2 Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each sample set; one set prior to analysis of the recovery samples, and the second set immediately following the analysis of the recovery samples. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

2.11.3 Method Differences

There were no method differences between SMV No. 14020.6109 and this procedure.

2.12 Evaluation of Precision, Accuracy, Specificity and Linearity

The accuracy was reported in terms of percent recovery of the low- and high-level recovery samples. Recoveries of 70 to 120% of nominal were considered acceptable, with no corrections made for procedural recoveries during the study. No more than 1/5 of the fortified recovery samples were excluded from the mean due to being out of specification. The precision was reported in terms of the standard deviation and relative standard deviation (RSD) for the retention time, the peak area quantitation, and the percent recovery values of the low- and high-level recovery samples for each analyte. The retention time should have an RSD of less than or equal to 2%. The RSD of the peak area based quantitation and of the recovery values should be less than or equal to 20%. Specificity of the method was determined by examination of the control samples for peaks at the same retention times as TCVP, TCBA, TCPEone, TCPEol and TCCEol which might interfere with the quantitation of analytes. Interferences with peak areas that are less than 50% at the limit of detection (LOD) are not considered significant. Linearity of the method was determined by the correlation coefficient (r^2), y-intercept and slope of the regression line. The signal response data should have an intercept close to zero and a correlation coefficient not less than 0.990. The precision of the method at the LOQ was reported in terms of the relative standard deviation or coefficient of variation of the observed recovery values.

2.13 Communications

Communications occurred with the Sponsor Monitor to discuss items such as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standard and control sample, and 3) pre-validation evaluation and method establishment including calibration curve linearity. A complete list of communications is maintained in the study raw data.

2.14 Time Required for Analysis

A normal batch of samples consists of 10 fortified and 2 unfortified samples, 1 matrix-match blank and 7 solvent standards (20 samples total) for each matrix. A single analyst completed a set of 20 samples in one working day (8 hours) with LC/MS/MS analysis performed overnight.

3.0 Calculations

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

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

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

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

where:

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