

Study Title

MRID# 514578-64

Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite
in Ground Water and Surface Water by LC-MS/MS

Test Guidelines

OCSPP 850.6100 (2012)
CA DPR SOP#QAQC012.00 (2017)

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1.0 INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of veratridine and its major metabolite, veratric acid in aqueous solutions, as well as establish storage stability of veratridine and veratric acid in aqueous solutions under refrigerated conditions. The analytical portion of this study was conducted (17 April to 15 June 2018). The method validation quantified the concentrations of veratridine and veratric acid present in recovery samples prepared in ground water and surface water with regards to linearity, accuracy, precision, a determination of the limit of quantitation (LOQ), limit of detection (LOD), and confirmation of analyte identification (specificity). The study also included a determination of the stability in aqueous solutions under refrigerated conditions.

The method was validated in ground water and surface water by fortification with veratridine and veratric acid at concentrations of approximately 0.10 (LOQ) and 1.0 (10X LOQ) $\mu\text{g/L}$.

Veratridine recovery samples were diluted with acetonitrile and purified reagent water and then further diluted into the calibration standard range with 20/80 acetonitrile/purified reagent water (v/v). Veratric acid recovery samples were acidified using 37% hydrochloric acid, extracted using dichloromethane, evaporated to dryness, and were additionally diluted into the calibration standard range with 20/80 acetonitrile/purified reagent water (v/v). All samples were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

The storage stability testing was performed by fortification in both ground water and surface water with veratridine and veratric acid at concentrations of 0.997 and 1.00 $\mu\text{g/L}$, respectively.

Duplicate samples of each matrix were extracted and analyzed at days 0, 7, 14, and 28 after storage at temperatures of 2 to 7 $^{\circ}\text{C}$.

The study was initiated on 11 April 2018, 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 and stability study was conducted at Smithers Viscient (SMV), located in Wareham,

Massachusetts. All original raw data, the protocol, and the final report produced during this study are stored in Smithers Viscient's archives at the above location.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this study followed those described in the Smithers Viscient protocol entitled “Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Ground Water and Surface Water by LC-MS/MS” ([Appendix 1](#)). The study was conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40 CFR 160 ([U.S. EPA, 1989](#)) and the OECD principles on GLP ([OECD, 1998](#)), and followed OCSP 850.6100 ([U.S. EPA, 2012](#)) and California Department Pesticide Regulation SOP# QAQC012.00 ([CA DPR, 2017](#)).

2.2 Test Substances

2.2.1 Veratridine

The test substance, veratridine, was received on 22 November 2016 from Sigma Aldrich, Inc., Milwaukee, Wisconsin. The following information was provided:

Name:	veratridine
Synonym:	3-veratrolylveracevine
Batch No.:	SLBF5557V
CAS No.:	71-62-5
Purity:	94.1% (Certificate of Analysis, Appendix 2)
Recertification Date:	12 April 2020

Upon receipt at Smithers Viscient, the test substance (SMV No. 8612) was stored in a freezer in the original container. Concentrations were adjusted for the purity of the test substance.

2.2.2 Veratric Acid

The test substance, 3,4-dimethoxybenzoic acid, was received on 15 January 2018 from Sigma-Aldrich, Inc., Milwaukee, Wisconsin. The following information was provided:

Name:	3,4-dimethoxybenzoic acid
Synonym:	Veratric acid
Batch No.:	BCBV0062
CAS No.:	93-07-2
Purity:	99.6 % (Certificate of Analysis, Appendix 2)
Retest Date:	15 January 2019

Upon receipt at Smithers Viscient, the test substance (SMV No. 9258) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

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

2.3 Reagents

- | | | |
|----|-----------------------------------|---|
| 1. | 0.1% Formic acid in water: | Fisher Chemical, reagent grade |
| 2. | 0.1% Formic acid in acetonitrile: | Fisher Chemical, reagent grade |
| 3. | Methanol: | EMD reagent grade |
| 4. | Acetonitrile: | EMD, reagent grade |
| 5. | Hydrochloric acid: | EMD, reagent grade |
| 6. | Dichloromethane: | EMD, reagent grade |
| 7. | Purified reagent water: | Prepared from a Millipore MilliQ Direct 8 water purification system (meets ASTM Type II requirements) |

Equivalent reagents/chemicals may be used and it is recommended to evaluate the reagents/chemical substitutes for interferences by taking a reagent blank through the entire analytical procedure.

2.4 Instrumentation and Laboratory Equipment

1. Instrument: MDS Sciex API 5000 mass spectrometer equipped with an ESI Turbo V ion source
Shimadzu SIL-20AHT autoinjector
Shimadzu DGU-20A3V vacuum degasser
Shimadzu DGU-20A5R vacuum degasser
Shimadzu LC-20AD solvent delivery pumps
Shimadzu CTO-20A column compartment
Shimadzu CBM-20A communications bus
Analyst 1.6.3 software for data acquisition
2. Balance: Mettler Toledo XSE205DU
3. Shaker Table: VWR 3500 Shaker Table
4. Centrifuges: Thermo Scientific Sorvall Legend XFR and Beckman Allegra X-12
5. Laboratory equipment: Positive displacement pipets, volumetric flasks, disposable glass vials, disposable glass pipets, centrifuge tubes, graduated cylinders, Whatman filter paper, amber vials with crimp caps, autosampler vials and amber glass bottles with Teflon-lined caps

Other equipment or instrumentation may be used in future testing but may require optimization to achieve the desired separation and sensitivity.

2.5 Test Matrices

The matrices used during this method validation were ground water and surface water.

Ground water information:

The ground water used during this method validation and respective stability portion of the study was well water collected from Research For Hire, Porterville, California (SMV Nos. R04-20-18-17 and R04-20-18-18, collected on 18 April 2018 and received on 20 April 2018, respectively). The well water was filtered prior to use using Whatman filter paper. The surface water was kept refrigerated in the dark prior to use. All documentation relating to the preparation, storage, and handling is maintained by Smithers Viscient. The ground water was characterized by Agvise Laboratories according to the table below:

Property	Result
pH:	8.0
Calcium:	48 ppm
Magnesium:	17 ppm
Sodium:	37 ppm
Hardness:	192 mg equivalent CaCO ₃ /L
Conductivity:	0.47 mmhos/cm
Sodium Adsorption Ratio (SAR):	1.18
Total Dissolved Solids:	220 ppm
Turbidity:	0.37 NTU

Surface water information:

Surface water used during this method validation was collected from Turner Ag Research, Inc., Yuba City, California (SMV No. R03-06-18-07, collected on 27 February 2018 and received on 6 March 2018). The surface water was kept refrigerated in the dark prior to use. All documentation relating to the preparation, storage, and handling is maintained by Smithers Viscient. The surface water was characterized by Agvise Laboratories according to the table below:

Property	Result
pH:	8.0
Calcium:	21 ppm
Magnesium:	18 ppm
Sodium:	42 ppm
Hardness:	130 mg equivalent CaCO ₃ /L
Conductivity:	0.37 mmhos/cm
Sodium Adsorption Ratio (SAR):	1.61
Total Dissolved Solids:	174 ppm
Turbidity:	58.2 NTU

2.6 Preparation of Liquid Reagent Solutions

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

A 20/80 acetonitrile/purified reagent water (v/v) liquid reagent solution was typically prepared by combining 100 mL of acetonitrile and 400 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes.

A 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v) autosampler needle wash solution was typically prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water. The solution was mixed well before use.

2.7 Preparation of Stock Solutions

The volumes and masses listed in this section were those used during each separate validation. For the storage stability study and any future testing, the actual volumes and masses used may be scaled up or down as necessary. Volumes and masses may be changed; however, the proportions must remain the same.

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
Test Substance						
8612-1F	0.0099	0.0093	Acetonitrile	10.0	932	Secondary stock solutions
Reference Substance						
9258A	0.0252	0.0251	Acetonitrile	25.0	1000	Secondary stock solutions

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
Test Substance							
8612-1F	932	0.250	25.0	Acetonitrile	8612-1F-1	9.32	Sub-stock solutions
Reference Substance							
9258A	1000	0.500	50.0	Acetonitrile	9258A-1	10.0	Sub-stock solutions

Sub-stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (µg/L)	Stock Use
8612-1F-1	9320	0.107	10.0	Acetonitrile	Tech Mix-Stk1	99.7 / 100 ^a	10X LOQ recovery samples and sub stock solution
9258A-1	10,000	0.100					
Tech Mix-Stk1	99.7 / 100 ^a	1.00	10.0	Acetonitrile	Tech Mix-Stk2	9.97 / 10.0 ^a	LOQ recovery samples
8612-1F-1	9320	0.107	50.0	Acetonitrile	Ana Mix-Stk1	19.9 / 1000 ^a	Sub-stock solution
9258A-1	10,000	5.00					
Ana Mix-Stk1	19.9 / 1000 ^a	0.500	10.0	Acetonitrile	Ana Mix-Stk2	0.995 / 50.0 ^a	Calibration standards

^a Concentrations are presented as veratridine/veratric acid.

All primary and secondary stock solutions of veratridine were stored in a freezer (approximately <0 °C) in amber glass bottles fitted with Teflon-lined caps. All primary and secondary stock solutions of veratric acid were stored refrigerated (2 to 7 °C) in amber glass bottles fitted with Teflon-lined caps. Sub-stock solutions were prepared fresh on the day of use and stored refrigerated for possible future use.

2.8 Preparation of Calibration Standards

Calibration standards were prepared in 20/80 acetonitrile/purified reagent water (v/v) by dosing with the 0.995/50.0 µg/L (veratridine/veratric acid) sub-stock solution to yield veratridine concentrations of 0.00498, 0.00796, 0.0119, 0.0199, 0.0398, 0.0597, and 0.0796 µg/L and veratric acid concentrations of 0.250, 0.400, 0.600, 1.00, 2.00, 3.00, and 4.00 µg/L.

2.9 Matrix Effect Investigation

In an effort to observe any potential matrix effects, prepared matrix blanks were fortified in triplicate and analyzed at each transition. These matrix-matched standards were compared to non-matrix matched standards fortified in triplicate at the same concentration. Standard solutions

used to assess possible matrix effects were prepared as described in the following tables.

2.9.1 Matrix-Matched Standards

Veratridine

Stock ID	Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL) ^a	Standard Concentration (µg/L)	Sample ID ^b
Ana Mix-Stk2	0.995	0.100	5.00	0.0199	V-MM-Std A / V-MM-Std D
		0.100	5.00	0.0199	V-MM-Std B / V-MM-Std E
		0.100	5.00	0.0199	V-MM-Std C / V-MM-Std F

^a Diluted with the final dilution of control samples 04-V, 05-V, and 06-V for the surface water matrix effect investigation and 22-V, 23-V, and 24-V for the ground water matrix effect investigation ([Section 2.10](#))

^b Sample IDs are presented as surface water / ground water validation.

Veratric Acid

Stock ID	Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL) ^a	Standard Concentration (µg/L)	Sample ID ^b
Ana Mix-Stk2	50.0	0.0162	1.00	0.810	VA-MM-Std A / VA-MM-Std D
		0.0162	1.00	0.810	VA-MM-Std B / VA-MM-Std E
		0.0162	1.00	0.810	VA-MM-Std C / VA-MM-Std F

^a Diluted with the final dilution of control samples 04-VA, 05-VA, and 06-VA for the surface water matrix effect investigation and 22-VA, 23-VA, and 24-VA for the ground water matrix effect investigation ([Section 2.10](#))

^b Sample IDs are presented as surface water / ground water validation.

2.9.2 Non-Matrix-Matched Standards

Veratridine

Stock ID	Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL) ^a	Standard Concentration (µg/L)	Sample ID ^b
Ana Mix-Stk2	0.995	0.100	5.00	0.0199	V-Std A / V-Std D
		0.100	5.00	0.0199	V-Std B / V-Std E
		0.100	5.00	0.0199	V-Std C / V-Std F

^a Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^b Sample IDs are presented as surface water / ground water validation.

Veratric Acid

Stock ID	Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL) ^a	Standard Concentration (µg/L)	Sample ID ^b
Ana Mix-Stk2	50.0	0.0162	1.00	0.810	VA-Std A / VA-Std D
		0.0162	1.00	0.810	VA-Std B / VA-Std E
		0.0162	1.00	0.810	VA-Std C / VA-Std F

^a Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^b Sample IDs are presented as surface water / ground water validation.

2.10 Sample Fortification and Preparation

The recovery samples were prepared in two different matrices (ground water and surface water) with veratridine and veratric acid at concentrations of approximately 0.10 (LOQ) and 1.0 (10X LOQ) µg/L. Prior to use, ground water was filtered through Whatman filter paper. Recovery samples for the two aqueous matrices were prepared separately (“de novo”) at these concentrations. Seven replicates were produced for the LOQ concentration and five replicates were produced for the 10X LOQ concentration. Following fortification, samples were divided into two aliquots, one aliquot (2.0 mL) was removed for the processing for veratridine, and the remaining aliquot was processed for veratric acid (18.0 mL). Five samples were left unfortified to serve as controls and were diluted in the same fashion as the LOQ concentration recovery samples (three controls of each matrix were utilized for matrix effects investigation). In addition, one reagent blank was prepared and processed in the same manner as the control samples. The preparation procedure for each separate matrix is outlined in the tables below.

Ground water recovery samples

Sample ID 14012.6138.	Sample Type	Stock Concentration (µg/L)	Fortification Volume (mL)	Final Volume (mL)	Fortified Concentration (µg/L)
19	Reagent Blank	NA ^a	NA	20.0 ^b	0.00
20, 21, 22, 23, & 24	Control	NA	NA	20.0	0.00
25, 26, 27, 28, 29, 30, & 31	LOQ	9.97 / 10.0 ^c	0.200	20.0	0.0997 / 0.100 ^c
32, 33, 34, 35, & 36	10X LOQ	99.7 / 100 ^c	0.200	20.0	0.997 / 1.00 ^c

^a NA = Not Applicable

^b Diluted with purified reagent water

^c Concentrations are presented as veratridine/veratric acid.

Surface water recovery samples

Sample ID 14012.6138.	Sample Type	Stock Concentration (µg/L)	Fortification Volume (mL)	Final Volume (mL)	Fortified Concentration (µg/L)
01	Reagent Blank	NA ^a	NA	20.0 ^b	0.00
02, 03, 04, 05, & 06	Control	NA	NA	20.0	0.00
07, 08, 09, 10, 11, 12, & 13	LOQ	9.97 / 10.0 ^c	0.200	20.0	0.0997 / 0.100 ^c
14, 15, 16, 17, & 18	10X LOQ	99.7 / 100 ^c	0.200	20.0	0.997 / 1.00 ^c

^a NA = Not Applicable

^b Diluted with purified reagent water

^c Concentrations are presented as veratridine/veratric acid.

2.11 Sample Processing

2.11.1 Veratridine

A 2.0 mL aliquot was removed from the fortified recovery sample, and transferred to a disposable glass vial. The first dilution with acetonitrile was performed by the addition of the reagent to the entire volume of the aqueous aliquot in the container in which it was transferred. The samples were subsequently diluted with purified reagent water. The 10X LOQ-level recovery samples were further diluted into the calibration standard range with 20/80 acetonitrile/purified reagent water (v/v) prior to analysis. The procedures are outlined in the tables below.

Ground water recovery samples

Sample ID 14012.6138.	Sample Type	Fortified Concentration (µg/L)	Sample Volume (mL)	Final Volume ^a (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
19-V	Reagent Blank	0.00	2.00	10.0	NA ^c	NA	5.00
20-V, 21-V, 22-V, 23-V, & 24-V	Control	0.00	2.00	10.0	NA	NA	5.00
25-V, 26-V, 27-V, 28-V, 29-V, 30-V, & 31-V	LOQ	0.0997	2.00	10.0	NA	NA	5.00
32-V, 33-V, 34-V, 35-V, & 36-V	10X LOQ	0.997	2.00	10.0	1.00	10.0	50.0

^a Diluted with acetonitrile (2.0 mL) and purified reagent water (6.0 mL)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

Surface water recovery samples

Sample ID 14012.6138.	Sample Type	Fortified Concentration (µg/L)	Sample Volume (mL)	Final Volume ^a (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
01-V	Reagent Blank	0.00	2.0	10.0	NA ^c	NA	5.00
02-V, 03-V, 04-V, 05-V, & 06-V	Control	0.00	2.0	10.0	NA	NA	5.00
07-V, 08-V, 09-V, 10-V, 11-V, 12-V, & 13-V	LOQ	0.0997	2.0	10.0	NA	NA	5.00
14-V, 15-V, 16-V, 17-V, & 18-V	10X LOQ	0.997	2.0	10.0	1.00	10.0	50.0

^a Diluted with acetonitrile (2.0 mL) and purified reagent water (6.0 mL)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

2.11.2 Veratric Acid

The remaining 18.0 mL of each fortified recovery sample were processed for veratric acid by first acidifying each sample with 0.100 mL of 37% hydrochloric acid and vortexed for 15 seconds. Next, a 10.0-mL aliquot of dichloromethane (DCM, CAS No. 75-09-2) was added to each sample and samples were vortexed, placed on a shaker table for 15 minutes at 250 rpm, and centrifuged at 1000 rpm for five minutes. An 8.0-mL aliquot of the bottom, DCM layer was then transferred to a 15 mL centrifuge tube. Care was taken to avoid sampling water with the DCM at this step. The DCM layer, in a 15.0-mL centrifuge tube, was placed in the N₂ evaporator and was evaporated under a stream of nitrogen at room temperature. The top, aqueous layer was then extracted again a second time as described above, an aliquot of the bottom, DCM layer (10 mL) was combined with the first extract already placed in the nitrogen evaporator, and the samples were evaporated to dryness. Samples were reconstituted and additionally diluted into the calibration standard range with 20/80 acetonitrile/purified reagent water (v/v). The 10X LOQ-level recovery samples were further diluted into the calibration standard range with 20/80 acetonitrile/purified reagent water (v/v) prior to analysis. The procedures are outlined in the tables below.

Ground water recovery samples

Sample ID 14012.6138.	Sample Type	Fortified Concentration (µg/L)	Sample Volume (mL)	Extract Volume ^a (mL)	Sample Volume of DCM (mL)	Final Volume ^b (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
19-VA	Reagent Blank	0.00	18.0	20.0	18.0	2.00	NA ^c	NA	0.123
20-VA, 21-VA, 22-VA, 23-VA, & 24-VA	Control	0.00	18.0	20.0	18.0	2.00	NA	NA	0.123
25-VA, 26-VA, 27-VA, 28-VA, 29-VA, 30-VA, & 31-VA	LOQ	0.100	18.0	20.0	18.0	2.00	NA	NA	0.123
32-VA, 33-VA, 34-VA, 35-VA, & 36-VA	10X LOQ	1.00	18.0	20.0	18.0	2.00	0.500	5.00	1.23

^a Extracted with dichloromethane (DCM)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

Surface water recovery samples

Sample ID 14012.6138.	Sample Type	Fortified Concentration (µg/L)	Sample Volume (mL)	Extract Volume (mL) ^a	Sample Volume of DCM (mL)	Final Volume ^b (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
01-VA	Reagent Blank	0.00	18.0	20.0	18.0	2.00	NA ^c	NA	0.123
02-VA, 03-VA, 04-VA, 05-VA, & 06-VA	Control	0.00	18.0	20.0	18.0	2.00	NA	NA	0.123
07-VA, 08-VA, 09-VA, 10-VA, 11-VA, 12-VA, & 13-VA	LOQ	0.100	18.0	20.0	18.0	2.00	NA	NA	0.123
14-VA, 15-VA, 16-VA, 17-VA, & 18-VA	10X LOQ	1.00	18.0	20.0	18.0	2.00	0.500	5.00	1.23

^a Extracted with dichloromethane (DCM)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

2.12 Analysis

2.12.1 Instrumental Conditions

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

LC parameters:

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

Gradient:	Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
	0.50	0.350	90.0	10.0
	3.00	0.350	0.00	100
	4.00	0.350	0.00	100
	4.10	0.350	90.0	10.0
	6.00	0.350	90.0	10.0
Run Time:	6.00 minutes			
Autosampler Wash Solvent:	30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)			
Column Temperature:	40 °C			
Sample Temperature:	10 °C			
Injection Volume:	20.0 µL			
Retention Times:	approximately 2.9 minutes (veratridine) approximately 2.7 minutes (veratric acid)			

MS parameters:

Instrument:	MDS Sciex API 5000 mass spectrometer
Ionization Mode:	Positive (+) ESI
Ion Spray Voltage:	5500 V
Scan Type:	MRM
Dwell Time:	150 milliseconds
Source Temperature:	600 °C
Curtain Gas:	20.0
Ion Source – Gas 1 / Gas 2:	60.0 / 60.0
Collision Gas:	10.0
Collision Cell Entrance Potential:	10.0
Declustering Potential:	166 (Veratridine) and 75.0 (Veratric Acid)
Resolution Q1/Q3:	Low/Low

Veratridine:

	Primary Transition	Confirmatory Transition
Q1/Q3 Masses (Da):	674.3/456.4	674.3/165.2
Collision Energy:	69.0	93.0
Collision Cell Exit Potential:	12.0	8.0

Veratric Acid:

	Primary Transition	Confirmatory Transition
Q1/Q3 Masses (Da):	183.1/139.1	183.1/124.1
Collision Energy:	17.0	25.0
Collision Cell Exit Potential:	17.0	16.0

Other instrumentation and settings 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.

2.12.2 Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each sample set. Calibration standards were interspersed among analysis of the recovery samples, every three to seven injections. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

2.13 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 reagent blank and control samples for peaks at the same retention times as veratridine and veratric acid which might interfere with the quantitation of the analytes. Linearity of the method was determined by the coefficient of determination (r^2), y-intercept, and slope of the regression line.

2.14 Limit of Quantitation (LOQ)

The method was validated at the LOQ. This was defined as the lowest fortification level successfully recovered. Blank values (reagent blanks and untreated control samples) did not exceed 30% of the LOQ.

2.15 Limit of Detection (LOD)

The LOD was calculated using the standard deviation of the average recovery in units of concentration of seven aliquots of the sample fortified at the LOQ, multiplied by the one-tailed t-statistic at the 99% confidence level for n-1 replicates ([CA DPR, 2017](#)). Representative calculations for the LOD can be found in [Section 4.0](#).

3.0 Storage Stability

3.1 Sample Fortification and Preparation

The storage stability samples were prepared at Smithers Viscient to yield concentrations of 0.997 and 1.00 µg/L veratridine and veratric acid, respectively in both ground water and surface water. Duplicate samples of each matrix were analyzed after approximately 0, 7, 14, and 28 days of refrigerated storage at 2 to 7 °C.

Eight samples of ground water and eight samples of surface water were fortified with the 99.7/100 µg/L veratridine/veratric acid sub-stock solution in individual 45-mL vials to obtain concentrations of 0.997/1.00 µg/L veratridine/veratric acid as presented in the tables below. In addition, three sets of contingency samples, in duplicate, were prepared in each matrix and were stored for possible future use.

Ground water storage stability samples

Sample ID C05-18-	Storage Interval	Stock Concentration ^a (mg/L)	Volume of Fortification (mL)	Sample Volume (mL)	Fortified Sample Concentration ^a (mg/L)
103	Day 0	99.7 / 100	0.200	20.0	0.997 / 1.00
104		99.7 / 100	0.200	20.0	0.997 / 1.00
105	Day 7	99.7 / 100	0.200	20.0	0.997 / 1.00
106		99.7 / 100	0.200	20.0	0.997 / 1.00
107	Day 14	99.7 / 100	0.200	20.0	0.997 / 1.00
108		99.7 / 100	0.200	20.0	0.997 / 1.00
109	Day 28	99.7 / 100	0.200	20.0	0.997 / 1.00
110		99.7 / 100	0.200	20.0	0.997 / 1.00
111, 112, 113, 114, 115, & 116	Contingency Samples	99.7 / 100	0.200	20.0	0.997 / 1.00

^a Concentrations are presented as veratridine/veratric acid.

Surface water storage stability samples

Sample ID C05-18-	Storage Interval	Stock Concentration ^a (mg/L)	Volume of Fortification (mL)	Sample Volume (mL)	Fortified Sample Concentration ^a (mg/L)
89	Day 0	99.7 / 100	0.200	20.0	0.997 / 1.00
90		99.7 / 100	0.200	20.0	0.997 / 1.00
91	Day 7	99.7 / 100	0.200	20.0	0.997 / 1.00
92		99.7 / 100	0.200	20.0	0.997 / 1.00
93	Day 14	99.7 / 100	0.200	20.0	0.997 / 1.00
94		99.7 / 100	0.200	20.0	0.997 / 1.00
95	Day 28	99.7 / 100	0.200	20.0	0.997 / 1.00
96		99.7 / 100	0.200	20.0	0.997 / 1.00
97, 98, 99, 100, 101, & 102	Contingency Samples	99.7 / 100	0.200	20.0	0.997 / 1.00

^a Concentrations are presented as veratridine/veratric acid.

Samples were stored refrigerated until the appropriate sampling time point, with the exception of day 0 samples, which were processed and analyzed on the day of preparation.

3.1.1 Quality Control (QC) Sample Preparation

Four QC samples were prepared for each analytical interval in each matrix. QC samples were fortified individually with the 99.7/100 µg/L (veratridine/veratric acid) sub-stock solution at concentrations of 0.499, 0.748, 0.997, and 0.997 µg/L as veratridine and at concentrations of

0.500, 0.750, 1.00, and 1.00 µg/L as veratric acid. A typical fortification scheme is presented in the table below:

Sample Type	Stock Concentration ^a (µg/L)	Fortification Volume (mL)	Final Volume ^b (mL)	Fortified Concentration ^a (µg/L)
QC #1 & #5	99.7 / 100	0.100	20.0	0.499 / 0.500
QC #2 & #6	99.7 / 100	0.150	20.0	0.748 / 0.750
QC #3 & #7	99.7 / 100	0.200	20.0	0.997 / 1.00
QC #4 & #8	99.7 / 100	0.200	20.0	0.997 / 1.00

^a Concentrations are presented as veratridine/veratric acid.

^b Diluted with ground water or surface water, as applicable.

In addition, one reagent blank in purified reagent water and one control in each matrix was also prepared and was processed in the same manner as LOQ samples during the validation study as presented in the table below:

Sample Type	Storage Interval	Stock Concentration ^a (mg/L)	Volume of Fortification (mL)	Sample Volume (mL)	Fortified Sample Concentration ^a (mg/L)
Reagent Blank	All Intervals	NA ^b	NA	20.0 ^c	0.00
Control	All Intervals	NA	NA	20.0	0.00

^a Concentrations are presented as veratridine/veratric acid.

^b NA = Not Applicable

^c Diluted with purified reagent water

3.2 Sample Processing

3.2.1 Veratridine

The veratridine storage stability samples were prepared and processed as detailed in [Section 2.10.1](#), with a 2.0-mL aliquot removed from each stability sample for the processing of veratridine. Two storage stability samples were processed at each sampling interval. The procedures are outlined in the table below.

Sample Type	Fortified Concentration (mg/L)	Sample Volume (mL)	Final Volume ^a (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
Reagent Blank	0.00	2.00	10.0	NA ^c	NA	5.00
Control	0.00	2.00	10.0	NA	NA	5.00
Storage Stability	0.997	2.00	10.0	1.00	10.0	50.0
Storage Stability	0.997	2.00	10.0	1.00	10.0	50.0
Quality Control	0.499	2.00	10.0	1.00	10.0	50.0
Quality Control	0.748	2.00	10.0	1.00	10.0	50.0
Quality Control	0.997	2.00	10.0	1.00	10.0	50.0
Quality Control	0.997	2.00	10.0	1.00	10.0	50.0

^a Diluted with acetonitrile (2.0 mL) and purified reagent water (6.0 mL)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

The storage stability samples were analyzed using the calibration standards detailed in [Section 2.7](#) above and the instrumental conditions from [Section 2.11.1](#).

3.2.2 Veratric Acid

The veratric acid storage stability samples were prepared and processed as detailed in [Section 2.10.2](#), with an 18.0-mL aliquot removed from each stability sample for the processing of veratric acid. Two storage stability samples were processed at each sampling interval. The procedures are outlined in the table below.

Sample Type	Fortified Concentration (µg/L)	Sample Volume (mL)	Extract Volume (mL) ^a	Sample Volume of DCM (mL)	Final Volume ^b (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
Reagent Blank	0.00	18.0	20.0	18.0	2.0	NA ^c	NA	0.123
Control	0.00	18.0	20.0	18.0	2.0	NA	NA	0.123
Storage Stability	1.00	18.0	20.0	18.0	2.0	0.500	5.00	1.23
Storage Stability	1.00	18.0	20.0	18.0	2.0	0.500	5.00	1.23
Quality Control	0.500	18.0	20.0	18.0	2.0	0.500	5.00	1.23
Quality Control	0.750	18.0	20.0	18.0	2.0	0.500	5.00	1.23
Quality Control	1.00	18.0	20.0	18.0	2.0	0.500	5.00	1.23
Quality Control	1.00	18.0	20.0	18.0	2.0	0.500	5.00	1.23

^a Extracted with dichloromethane (DCM)

^b Diluted with 20/80 acetonitrile/purified reagent water (v/v)

^c NA = Not Applicable

The storage stability samples were analyzed using the calibration standards detailed in [Section 2.7](#) above and the instrumental conditions from [Section 2.11.1](#).

4.0 CALCULATIONS

A calibration curve was constructed by plotting the analyte concentration ($\mu\text{g/L}$) of the calibration standards against the peak area of the analyte in the calibration standards. A 1/x-weighted linear regression was used to quantify the recovery samples. The equation for the line including the slope and intercept was generated using standard software (e.g., Microsoft Excel). The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of test substance in each recovery sample was calculated using the slope and intercept from the linear regression analysis, the detector response, and the dilution factor of the recovery sample. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

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

$$(2) \quad \text{DC (x)} = \frac{(y - b)}{m}$$

$$(3) \quad A = \text{DC} \times \text{DF}$$

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 ($\mu\text{g/L}$) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample volume)
A	=	analytical result ($\mu\text{g/L}$), concentration in the original sample

The LOD (aka MDL) was calculated using the following equation ([CA DPR, 2017](#)):

$$(4) \quad \text{LOD} = t_{0.99} \times \text{SD}$$

where:

$t_{0.99}$	=	One-tailed t-statistic at the 99% confidence level for n-1 replicates (i.e., 3.143 for seven replicates)
SD	=	Standard deviation of the detected concentrations of n samples spiked at the estimated LOQ
LOD	=	Limit of detection for the analysis



TEST PROTOCOL

Title: Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Ground Water and Surface Water by LC-MS/MS

Data Requirement(s): OCSP 850.6100 and California Department of Pesticide Regulation SOP#QAQC0.12.00

Test Substances:

Name:	Veratridine
Purity:	90.6%
Batch or Lot #:	SLBF5557V
Name:	3,4-Dimethoxybenzoic acid (Veratric Acid)
Purity:	99.6%
Batch or Lot #:	BCBV0062

Study Sponsor: Steve Klaysmat
Address: McLaughlin Gromley & King, MGK®
8810 Tenth Avenue North
Minneapolis, MN 55427

Study Monitor: Mark Lenz, Ph.D.
Email / Phone Number: mlenz@exponent.com Tel: (913) 213-9519

Sponsor Protocol/Project No. (when applicable): NA

Testing Facility: Smithers Viscient,
790 Main Street
Wareham, Massachusetts 02571

Study Director: Johnson I. Jutson, Ph.D.

Smithers Viscient Study No.: 14012.6138

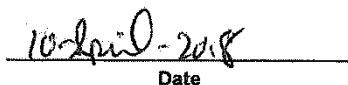
Test Concentrations: 0.10 and 1.0 µg/L

Proposed Experimental Dates

Start: April 17, 2018
Termination: May 29, 2018



Sponsor Approval



Date



Study Director Signature



Study Initiation Date

Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite Veratric Acid in Ground Water and Surface Water by LC-MS/MS

1.0 INTRODUCTION

The purpose of this study is to validate an analytical method used to determine the content of veratridine and its major metabolite in two aqueous matrices (ground water and surface water) by LC-MS/MS. The analytical method will be validated with regards to specificity, linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), method detection limit (MDL), and confirmation of identification.

2.0 JUSTIFICATION OF THE TEST SYSTEM

This study is conducted to support the registration of the test substance.

The method validations described in this protocol are designed to conform to EPA guideline OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. The study will be conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40CFR160 and as compatible with OECD principles on GLP.

3.0 TEST SUBSTANCE

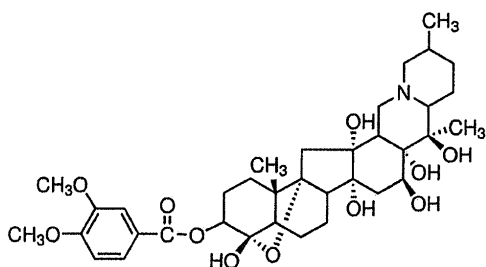
3.1 Test Substance

Upon arrival at Smithers Visient, the test substance (also the reference substance) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

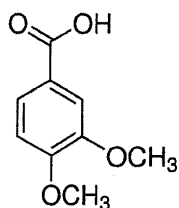
Each test and reference substance will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

3.2 Reference Standards

Residues of veratridine and veratric acid will be determined in surface water and groundwater. Current available information on the analytes is summarized below.



Name:	Veratridine
Synonym:	3-veratroylveracevine
Batch/Lot No.:	SLBF5557V
CAS No.:	71-62-5
Purity:	To be added by amendment
Recertification Date:	To be added by amendment



Name:	3,4-dimethoxybenzoic acid
Synonym:	Veratric acid
Batch/Lot No.:	BCBV0062
CAS No.:	93-07-2
Purity:	99.6%
Expiration Date:	01/15/2019

3.3 Test Matrices

3.3.1 Ground Water

The ground water used for the method validation will be collected from California, if feasible. The ground water used for the method validation will be filtered well water prepared by filtering to remove any potential organic contaminants. All documentation relating to the preparation, storage and handling will be maintained by Smithers Viscient.

3.3.2 Surface Water

The surface water used for the method validation will be collected from California, if feasible. All documentation relating to the collection, preparation, storage and handling will be maintained by Smithers Viscient.

3.4 Reagents

Highly pure reagents will be used throughout the study. The actual reagent grade will be depending on the manufacturer's designation. Generally, these reagents will have grades, such as high purity solvent, ACS grade, or Select. The reagents used are recorded along with test chemical information at the time of preparation.

4.0 VALIDATION DESIGN

The test design will consist of two aqueous matrices (ground water and surface water) fortified with each test substance at two concentrations with seven replications at the target LOQ and five replicates at 10x LOQ level for each matrix. The procedural blank will be reagent blank without matrix. The control matrix for the validation will be untreated matrix representing ground water or surface water. The validation study levels (approximate concentrations) for test substance are:

- | | | |
|----|--------------------------------------|-----------|
| 1. | Procedural blank-reagent blank | 0.0 µg/L |
| 2. | Matrix blank-control matrix | 0.0 µg/L |
| 3. | Control matrix fortified at LOQ | 0.10 µg/L |
| 4. | Control matrix fortified at 10 x LOQ | 1.0 µg/L |

4.1 Accuracy and Precision

The accuracy of the analytical method will be determined by applying the method to seven samples at the LOQ and five samples at 10X LOQ for each test substance. Accuracy will be reported as the mean recovery at each fortification level. Mean recoveries in the range 70 – 120% of nominal concentrations of the target analyte in the fortified samples will be considered acceptable.

The precision will be calculated for the fortified samples in terms of the standard deviation (SD) and relative standard deviation (RSD or coefficient of variation (CV)) calculated for the retention time, peak area based quantitation (i.e., µg/L), and the observed recovery values. The retention time should have a RSD of less than or equal to 2%. The RSD of the peak area based quantitation (i.e., µg/L) should be less than or equal to 20% per level. The RSD of the recovery values should be less than or equal to 20% per level as well.

4.2 Specificity

The specificity of the method will be determined by applying the method to the appropriate number of reagent blank and control matrix samples (n=2). Chromatograms will be obtained for the control samples and examined for peaks that might interfere with the quantitation of the analyte(s) peak of interest. Peaks attributable to the test substance(s) should be sufficiently resolved from any peaks found in the samples of control matrix to enable quantification. Blank values (including

procedural blanks and untreated samples) should not exceed 30% of the LOQ. If this is exceeded, detailed justification is required.

4.3 Regression Analysis

Quantitative analysis will be achieved with the aid of a calibration curve. The calibration curve will be constructed using a minimum of five analytical standards and will extend over a range appropriate to the lowest and highest nominal concentrations of the target analyte in relevant analytical solutions $\pm$ at least 20%.

The calibration data will be subjected to regression analysis; a plot of analyte concentration versus detector response will be included in the report along with the correlation coefficient (r) and the equation describing the curve. The linearity of the detector response will be assessed according to the strength of the correlation coefficient: this should be ≥ 0.995 (or coefficient of determination, $r^2 \geq 0.990$). If non-linear calibration is used an explanation will be provided.

4.4 Limits of Quantitation (LOQ)

The method will be validated at the limit of quantitation (LOQ). This will be defined as the lowest fortification level. Blank values (reagent blanks and untreated control samples) should not exceed 30% of the LOQ. If this is exceeded, it will be discussed with the Sponsor and detailed justification provided prior to processing.

4.5 Limits of Detection (LOD)

The Limits of Detection (LOD) will be calculated using the standard deviation of the average recovery in units of concentration of seven aliquots of the sample fortified at the LOQ multiplied by one-tailed t-statistic at the 99% confidence level for $n-1$ replicates (CA DPR, 2017).

4.6 Matrix Effects Determination

Determination of LC-MS/MS matrix effects will be evaluated through the assessment of solvent-based and matrix-matched calibration standards for both primary and confirmatory transitions. Matrix effects should be evaluated at the LOQ level for each test substance. Only if experiments clearly demonstrate that matrix effects are not significant (i.e. $<20\%$), calibration with standards in solvent may be used.

4.7 Confirmatory Analyses

Unequivocal identification of the target analyte will be achieved by LC/MS-MS using a primary quantitation ion and secondary quantitation/confirmatory ion. All of the required elements need to be met for this confirmatory method with full method validation results generated for both transitions. For triple-quad MS method, the confirmation method would be where confirmatory (secondary) product ion will be used for quantification. The confirmatory method analysis will also adhere to the aforementioned method specifications (Sections 4.1-4.6 above)

4.8 Storage Stability

A minimum of 28-day storage stability will be established in both soil and sediment matrices. Surface and groundwater matrices will be fortified at 10X LOQ level and stored in a refrigerator between 2 to 7 °C for the duration of the test. Two replicate samples will be analyzed at four

sampling intervals (For example, Day 0, Day7, Day 14 and Day 28) to validate residue's rate of decomposition in respective matrices. Analysis will be conducted with validated analytical method with acceptable recovery range of 70-120% with RSD of <20%.

5.0 PROCEDURE FOR THE IDENTIFICATION OF THE TEST SYSTEM

The test system will be defined as the fortified recovery samples. The fortified recovery samples will be labeled as defined in section 4.0 and each sample replicate will be assigned a unique identifier. Processing of fortified recovery samples will be performed at a lab station labeled with the study number.

6.0 CONTROL OF BIAS

Bias will be effectively controlled through techniques such as, but not limited to, preparation of replicate samples and replicate analysis.

7.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

8.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. A single copy of the draft report will be submitted to the Sponsor for review. The report will be finalized according to standard operating procedures. The final report will meet the formatting requirements of EPA's PR Notice 2011-3. All reports will include, but will not be limited to reporting requirements presented in Ecological Effects Test Guidelines OCSPP 850.6100 (U.S. EPA, 2012) and California DPR Guide for Analytical Method Development (CA DPR, 2017) and the following information:

- The report and project numbers from Smithers Viscient and Sponsor Study number (if any).
- Laboratory and site, dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director and Principal Investigator.
- Identification of the test substance including chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, degree of purity of test substance (percent test chemical) (Sponsor supplied, if available).

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- A full description of the experimental design and procedures followed and a description of the test equipment used.
- The determined specificity, linearity, accuracy, precision, LOQ, LOD and MDL, and confirmation of identification.
- The mathematical equations and statistical methods used in generating and analyzing the data as well as calculations using these equations. Tabular and graphical representations (if appropriate) of the data.
- Description of any problems experienced and how they were resolved.
- Good Laboratory Practice (GLP) Compliance Statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of raw data and report.
- A copy of the study protocol and study amendments, if any.

9.0 PROTOCOL AMENDMENTS

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any.

10.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the U.S. Environmental Protection Agency's Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160) and as compatible with OECD Principles of Good Laboratory Practice (OECD, 1998)

11.0 REFERENCES

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

U.S. EPA. 2011. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 7/1/2011; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2011. Pesticide Registration (PR) Notice 2011-3 Standard Format for Data Submitted Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Certain

Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA). US Environmental Protection Agency Office of Pesticide Programs. November 30, 2011.

U.S. EPA, 2012. Office of Chemical Safety and Pollution Prevention. Ecological Effects Guideline, OCSPP 850.6100. Environmental Chemistry Methods and Associated Independent Laboratory Validation. EPA 712-C-001. January 2012. U.S. Environmental Protection Agency, Washington, D.C.

CA DPR, 2017. California Department of Pesticide Regulation. 2017. Guide for Analytical Method Development, SOP#QAQC0.12.0. January 2017. California Department of Pesticide Regulation, Sacramento, CA.



PROTOCOL AMENDMENT

Amendment No.: 1

Protocol Title: Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Ground Water and Surface Water by LC-MS/MS

Study Sponsor: McLaughlin Gormley & King, MGK®

Test Substance(s): Veratridine & 3,4-Dimethoxybenzoic acid (Veratric Acid)

Smithers Viscient Study No.: 14012.6138

Amendment:

Protocol Currently States:

Test Substances: Name: Veratridine
Purity: 90.6%

Address: McLaughlin Gormley & King, MGK®
8810 Tenth Avenue North
Minneapolis, MN 55427

Update Protocol to State:

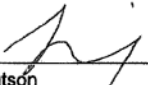
Test Substances: Name: Veratridine
Purity: 94.1%

Address: McLaughlin Gormley & King, MGK®
8810 Tenth Avenue North
Minneapolis, MN 55427

Reason for Change: The purpose of this amendment is to correct typographical error in the sponsor's name and to update the purity based on the updated purity.

None of the above changes will have a negative impact on the study.

Approval Signatures:



Johnson I. Jutson
Smithers Viscient Study Director

18 May 2018
Effective Date



Study Sponsor Representative

22 May - 2018
Date



PROTOCOL AMENDMENT

Amendment No.: 2

Protocol Title: Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Ground Water and Surface Water by LC-MS/MS

Study Sponsor: McLaughlin Gormley & King, MGK®

Test Substance(s): Veratridine & 3,4-Dimethoxybenzoic acid (Veratric Acid)

Study No.: 14012.6138

Amendment:

Section 2.0:

Justification of the Test System

This study is conducted to support the registration of the test substance.

The method validations described in this protocol are designed to conform to EPA guideline OCSP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. The study will be conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40CFR160 and as compatible with OECD principles on GLP.

Revise protocol to state:

The study will be conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40CFR160 and as accepted by OECD principles of Good Laboratory Practice (OECD, 1998).

Reason for Change: To clarify language of OECD principles according to GLPs.

Section 3.2:

Reference Standards

Name:	Veratridine
Synonym:	3-veratroylveracevine
Batch/Lot No.:	SLBF5557V
CAS No.:	71-62-5
Purity:	To be added by amendment
Recertification Date:	To be added by amendment

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Smithers Viscient LLC
www.smithersviscient.com

790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

Update protocol to state:

Purity: 94.1%
Recertification Date: 12 April 2020

Reason for Change: To update purity and recertification date.

Section 4.8:

Storage Stability

A minimum of 28-day storage stability will be established in both soil and sediment matrices.

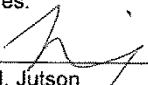
Revise protocol to state:

A minimum of 28-day storage stability will be established in both ground water and surface water matrices.

Reason for Change: To correct the matrices used for storage stability.

None of the above changes will have a negative impact on the study.

Approval Signatures:

 _____ Johnson I. Jutson Smithers Viscent Study Director	<u>20 Nov 2019</u> Effective Date
 _____ Sponsor Approval	<u>20-Nov-2019</u> Date