Analytical method for Veratridine, a component of Sabadilla Alkaloids, and its major degradate Veratric acid in water

Reports: ECM: EPA MRID No. 51457864. Jutson, J.I. 2018. Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Ground Water and Surface Water by LC-MS/MS. Smithers Viscient Study No.: 14012.6138. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by MGK® Minneapolis, Minnesota; 132 pages. Final report issued November 28, 2018.

ILV: EPA MRID No. 51457865. Schoenau, E.A. 2019. Independent Laboratory Validation of the Residue Analytical Methods for the Determination of Veratridine and its Major Metabolite (Veratric Acid) in Soil, Sediment, and Water by LC-MS/MS (Smithers Viscient Studies: 14012.6138 and 14012.6139). GPL Study No.: 180816. Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California, and sponsored and submitted by McLaughlin Gormley King Co. (MGK), Minneapolis, Minnesota; 190 pages. Final report issued December 2, 2019.

Document No.: MRIDs 51457864 & 51457865

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1989), which are accepted by the OECD GLP standards (1998), except that the veratric acid test substance was non-GLP characterized prior to use in the study (p. 3 of MRID 51457864). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement.
ILV: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1989), except that the veratric acid test substance was non-GLP characterized prior to use in the study (p. 3 of MRID 51457865). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement. Sponsor/Submitter signatures were dated January 30, 2020 and April 16, 2021 (pp. 2-3, 5).

Classification: This analytical method is classified as **Acceptable**. However, the ILV suggested that the ECM method should be updated with precautions for the use of glass laboratory vessels. And the LOD was not reported in the ILV.

PC Code:	002203		
Reviewer:	A'ja Duncan, Ph.D., Chemist	Signature: Date: 6/22/22	AJA DUNCAN Digitally signed by AJA DUNCAN Date: 2022.06.22 13:10:23 -04'00'
	Ibrahim Abdel-Saheb, Ph.D., Environmental Scientist	Signature: Date: 6/22/22	IBRAHIM ABDEL SAHEB Digitally signed by IBRAHIM ABDEL SAHEB Date: 2022.06.22 09:36:03 -04'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	Lisa Muto 11/10/2021
	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	Marysamuel 11/10/2021

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Smithers Viscient Analytical Method 14012.6138, is designed for the quantitative determination of veratridine and its major degradate veratric acid at 0.0997 µg/L and 0.10 µg/L, respectively, in water using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water (65 µg/L; MRID 51457881). The reported ILV LOQ for veratridine (0.10 µg/L) differed slightly from the reported ECM LOQ for veratridine due to the ECM adjustment for test material purity. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ILV reported method LOQ for both analytes in water (0.10 µg/L).

The ECM and ILV validated the method using different characterized surface and ground water matrices. The ILV validated Smithers Viscient Analytical Method 14012.6138 with the first trial for veratridine and veratric acid in both water matrices with minor modifications to the sample processing of the final extracts for veratric acid analysis (including the 10-fold dilution) and with insignificant modifications to the analytical parameters and equipment. The extraction modification for veratric acid was made to the method due to low recoveries in the first injections of ground and surface water. The low recoveries were assumed to be due to labware interference. Following the ILV suggestion, the ECM method should be "updated to specify that water samples should be extracted in glass" (p. 34). Additionally, the ECM method should be updated to advise the use of a 10-fold dilution for veratric acid analysis if glass laboratory extraction vessels cannot be used. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for veratridine and veratric acid.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Veratridine	51457864[1]	51457865[2]		Water	28/11/2018	McLaughlin Gormley King Co. (MGK)	LC/MS/MS	0.10 µg/L (ILV) [3]
Veratric acid								0.10 µg/L

1 In the ECM, ground (well) water (SMV Nos. R04-20-18-17 & R04-20-18-18; collected on April 18, 2018; pH 8.0, hardness 192 mg/L $CaCO_3$, conductivity 0.47 mmhos/cm, total dissolved solids 220 ppm), which was obtained from Research For Hire, Porterville, California, and surface water (SMV No. R03-06-18-07; collected on February 27, 2018; pH 8.0, hardness 130 mg/L $CaCO_3$, conductivity 0.37 mmhos/cm, total dissolved solids 174 ppm) which was obtained from Turner Ag Research, Inc., Yuba City, California, were used in the study (pp. 17-18 of MRID 51457864). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, ground (well) water (Sample ID: WELLW-0612190B; collected on June 12, 2019; pH 7.9, hardness 209 mg/L $CaCO_3$, conductivity 0.49 mmhos/cm, total dissolved solids 356 ppm) and surface (irrigation canal) water (Sample ID: HERNDON-39-061219-B; collected on June 12, 2019; pH 6.9, hardness 12 mg/L $CaCO_3$, conductivity 0.07 mmhos/cm, total dissolved solids 22 ppm) were obtained from Fresno, California, and used in the study (pp. 22-23; Appendix D, pp. 81-82 of MRID 51457865). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

3 The reported ILV LOQ for veratridine differed slightly from the reported ECM LOQ for veratridine (p. 33 of MRID 51457864; pp. 33 of MRID 51457865). The difference was due to an ECM adjustment for test material purity (pp. 15, 19, 22-23 of MRID 51457864; see Reviewer's Comment #1).

I. Principle of the Method

Samples (20.0 mL, final volume) of water were fortified with 200 µL of 0.0997 mg/L or 0.997 mg/L veratridine fortification solutions and 200 µL of 0.100 mg/L or 1.00 mg/L veratric acid fortification solutions, as necessary (pp. 22-25 of MRID 51457864). For veratridine analysis, a 2-mL aliquot of the fortified sample was transferred to a disposable glass vial then acetonitrile (2 mL) and purified reagent water (6 mL) were added. The LOQ samples were analyzed directly by LC/MS/MS. For the 10×LOQ samples, a 1.00-mL aliquot was diluted to 10.0 mL with acetonitrile:purified reagent water (20:80, v:v) prior to analysis. For veratric acid, the remaining 18 mL of the original fortified sample was acidified with 0.100 mL of 37% HCl and vortexed for 15 seconds. The acidified sample was extracted twice with dichloromethane (10 mL x 2) via shaking on a shaker table for 15 minutes at 250 rpm and centrifuging for 5 minutes at 1000 rpm. The method noted that care was taken to avoid sampling water with the dichloromethane during separation. The combined dichloromethane extracts were evaporated to dryness un a stream of nitrogen at room temperature. Samples were reconstituted to 2 mL using acetonitrile:purified reagent water (20:80, v:v). The LOQ samples were analyzed directly by LC/MS/MS. For the 10×LOQ samples, a 0.50-mL aliquot was diluted to 5.00 mL with acetonitrile:purified reagent water (20:80, v:v) prior to analysis.

Samples were analyzed for veratridine and veratric acid using a Shimadzu LC-20AD HPLC coupled to an MDS Sciex API 5000 mass spectrometer equipped with an ESI Turbo V interface

in the positive ion, multiple reaction monitoring (MRM) mode (pp. 17, 25-26 of MRID 51457864). The following LC conditions were used: Waters XBrdige BEH C18 column (2.1 mm x 50 mm, 2.5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.50 min. 90.0:10.0, 3.00-4.00 min. 0.00:100.0, 4.10-6.00 min. 90.0:10.0], MS temperature 600°C, and injection volume of 20.0 µL. Expected retention times were *ca*. 2.9 and 2.7 minutes for veratridine and veratric acid, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 674.3→456.4 and *m/z* 674.3→165.2 for veratridine, and *m/z* 183.1→139.1 and *m/z* 183.1→124.1 for veratric acid. Solvent-based standards were used for quantitation (p. 33).

The ILV performed the ECM method, Smithers Viscient Analytical Method 14012.6138, as written, except for the fact that the dichloromethane extracts were evaporated using a TurboVap under nitrogen and final extracts for veratric acid analysis were vortexed (1 minute), sonicated (10 minutes), and diluted 10-fold with acetonitrile:water (20:80, v:v) prior to analysis, and for insignificant modifications to the analytical parameters and equipment (pp. 15-26; Appendix C, pp. 78-79 of MRID 51457865). Samples were analyzed for veratridine and veratric acid using Shimadzu LC-20AD XR HPLC coupled to a Sciex API 5000 mass spectrometer equipped with an ESI interface in the positive ion, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except that injection volume was 25 µL. Expected retention times were *ca*. 2.9 and 2.3-2.6 minutes for veratridine and veratric acid, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 674.4→456.4 and *m/z* 674.4→165.1 for veratridine, and *m/z* 183.1→139.1 and *m/z* 183.1→124.1 for veratric acid; the monitored ion transitions of the ILV were similar to those of the ECM. The extraction modification for veratric acid was made to the method due to low recoveries in the first injections of ground and surface water (p. 24). The low recoveries were assumed to be due to labware interference (p. 26). The ILV could not employ the glass centrifuge tubes which were advised by the ECM personnel, but the 10-fold extraction of the original extracts resulted in acceptable recoveries. Following the ILV suggestion, the ECM method should be "updated to specify that water samples should be extracted in glass" (p. 34). Additionally, the ECM method should be updated to advise the use of a 10-fold dilution for veratric acid analysis if glass laboratory extraction vessels cannot be used.

The Limit of Quantification (LOQ) in ground and surface water was reported as 0.0997 µg/L and 0.10 µg/L for veratridine and veratric acid, respectively, in the ECM and 0.10 µg/L µg/L for veratridine and veratric acid in the ILV (pp. 27-28, 32-33, 35-37 of MRID 51457864; p. 33 of MRID 51457865). The Limit of Detection (LOD) in ground and surface water was calculated as 0.00834-0.0150 µg/L and 0.0141-0.0194 µg/L for veratridine and veratric acid, respectively, in the ECM. The LOD was not reported for veratridine and veratric acid in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51457864): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of veratridine in two water matrices at the LOQ (0.0997 µg/L) and 10×LOQ (0.997 µg/L; Tables 1-8, pp. 41-48). Mean recoveries and RSDs met requirements for analysis of veratric acid in two water matrices at the LOQ (0.10 µg/L) and 10×LOQ (1.00 µg/L). Recovery results of the quantitative and confirmatory ion transitions were comparable. The ground (well) water (SMV Nos. R04-20-18-17 & R04-20-18-18; collected on April 18, 2018; pH 8.0, hardness 192 mg/L $CaCO_3$, conductivity 0.47 mmhos/cm, total dissolved solids 220 ppm), which was obtained from Research For Hire, Porterville, California, and surface water (SMV No. R03-06-18-07; collected on February 27, 2018; pH 8.0, hardness 130 mg/L $CaCO_3$, conductivity 0.37 mmhos/cm, total dissolved solids 174 ppm) which was obtained from Turner Ag Research, Inc., Yuba City, California, were used in the study (pp. 17-18 of MRID 51457864). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 51457865): Mean recoveries and RSDs met requirements for analysis of veratridine and veratric acid in two water matrices at the LOQ (0.10 µg/L) and 10×LOQ (1.00 µg/L; Tables 9-20, pp. 45-56). Veratric acid recovery results are reported based on the re-analysis results of the samples; several original recovery results were <70% at the LOQ. Recovery results of the quantitative and confirmatory ion transitions were comparable, except for the LOQ analysis of Veratric acid in both water matrices where the quantitation ion analysis results were significantly lower than those of the confirmation ion analysis. The ground (well) water (Sample ID: WELLW-0612190B; collected on June 12, 2019; pH 7.9, hardness 209 mg/L $CaCO_3$, conductivity 0.49 mmhos/cm, total dissolved solids 356 ppm) and surface (irrigation canal) water (Sample ID: HERNDON-39-061219-B; collected on June 12, 2019; pH 6.9, hardness 12 mg/L $CaCO_3$, conductivity 0.07 mmhos/cm, total dissolved solids 22 ppm) were obtained from Fresno, California, and used in the study (pp. 22-23; Appendix D, pp. 81-82 of MRID 51457865). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

The method, Smithers Viscient Analytical Method 14012.6138, was validated by the ILV with the first trial for veratridine and veratric acid in both water matrices with minor modifications to the sample processing of the final extracts for veratric acid analysis (including the 10-fold dilution) and with insignificant modifications to the analytical parameters and equipment (pp. 15-26). The extraction modification for veratric acid was made to the method due to low recoveries in the first injections of ground and surface water (p. 24). The low recoveries were assumed to be due to labware interference (p. 26). The ILV could not employ the glass centrifuge tubes which were advised by the ECM personnel, but the 10-fold extraction of the original extracts resulted in acceptable recoveries. Following the ILV suggestion, the ECM method should be "updated to specify that water samples should be extracted in glass" (p. 34). Additionally, the ECM method should be updated to advise the use of a 10-fold dilution for veratric acid analysis if glass laboratory extraction vessels cannot be used.

Table 2. Initial Validation Method Recoveries for Veratridine and Veratric acid in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion transition						
Veratridine	0.0997 (LOQ)	7	94.4-102	97.9	2.69	2.75
	0.997	5	93.0-102	97.4	3.34	3.43
Veratric acid	0.10 (LOQ)	7	87.6-108	98.6	6.18	6.27
	1.00	5	100-109	104	3.87	3.72
Confirmation ion transition						
Veratridine	0.0997 (LOQ)	7	96.5-110	99.9	4.88	4.88
	0.997	5	94.2-108	102	5.24	5.14
Veratric acid	0.10 (LOQ)	7	91.8-110	100	5.38	5.37
	1.00	5	103-110	107	2.55	2.38
Surface Water						
Quantitation ion transition						
Veratridine	0.0997 (LOQ)	7	90.5-98.1	94.7	2.68	2.83
	0.997	5	91.3-96.5	93.5	2.17	2.32
Veratric acid	0.10 (LOQ)	7	97.5-112	104	4.89	4.69
	1.00	5	104-116	108	4.56	4.21
Confirmation ion transition						
Veratridine	0.0997 (LOQ)	7	87.4-99.8	92.0	4.81	5.22
	0.997	5	87.1-98.4	95.0	4.70	4.95
Veratric acid	0.10 (LOQ)	7	98.8-112	105	4.47	4.25
	1.00	5	105-116	109	4.83	4.41

Data (uncorrected results, p. 32) were obtained from Tables 1-8, pp. 41-48 of MRID 51457864. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 The ground (well) water (SMV Nos. R04-20-18-17 & R04-20-18-18; collected on April 18, 2018; pH 8.0, hardness 192 mg/L $CaCO_3$, conductivity 0.47 mmhos/cm, total dissolved solids 220 ppm), which was obtained from Research For Hire, Porterville, California, and surface water (SMV No. R03-06-18-07; collected on February 27, 2018; pH 8.0, hardness 130 mg/L $CaCO_3$, conductivity 0.37 mmhos/cm, total dissolved solids 174 ppm) which was obtained from Turner Ag Research, Inc., Yuba City, California, were used in the study (pp. 17-18). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 674.3→456.4 and *m/z* 674.3→165.2 for veratridine, and *m/z* 183.1→139.1 and *m/z* 183.1→124.1 for veratric acid.

Table 3. Independent Validation Method Recoveries for Veratridine and Veratric acid in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion transition						
Veratridine	0.10 (LOQ)	5	100-104	102	1.67	1.64
	1.00	5	90.0-102	94.7	4.49	4.74
Veratric acid[3]	0.10 (LOQ)	5	73.5-79.7	76.0	2.43	3.20
	1.00	5	81.3-88.0	84.8	3.04	3.58
Confirmation ion transition						
Veratridine	0.10 (LOQ)	5	99.0-103	101	1.48	1.47
	1.00	5	91.5-98.0	95.1	2.38	2.50
Veratric acid[3]	0.10 (LOQ)	5	80.6-96.8	86.8	6.80	7.83
	1.00	5	87.9-93.0	90.3	2.09	2.31
Surface Water						
Quantitation ion transition						
Veratridine	0.10 (LOQ)	5	91.5-96.0	94.2	1.79	1.90
	1.00	5	88.0-93.0	90.2	1.82	2.02
Veratric acid[3]	0.10 (LOQ)	5	75.1-89.5	79.3	6.19	7.81
	1.00	5	81.6-85.8	83.9	1.72	2.05
Confirmation ion transition						
Veratridine	0.10 (LOQ)	5	93.0-99.5	96.4	2.63	2.73
	1.00	5	87.5-96.0	92.8	3.67	3.95
Veratric acid[3]	0.10 (LOQ)	5	80.8-103	90.2	8.69	9.63
	1.00	5	83.4-90.1	87.7	3.03	3.45

Data (uncorrected results, pp. 27-29) were obtained from Tables 9-20, pp. 45-56 of MRID 51457865. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 The ground (well) water (Sample ID: WELLW-0612190B; collected on June 12, 2019; pH 7.9, hardness 209 mg/L $CaCO_3$, conductivity 0.49 mmhos/cm, total dissolved solids 356 ppm) and surface (irrigation canal) water (Sample ID: HERNDON-39-061219-B; collected on June 12, 2019; pH 6.9, hardness 12 mg/L $CaCO_3$, conductivity 0.07 mmhos/cm, total dissolved solids 22 ppm) were obtained from Fresno, California, and used in the study (pp. 22-23; Appendix D, pp. 81-82). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 674.4→456.4 and *m/z* 674.4→165.1 for veratridine, and *m/z* 183.1→139.1 and *m/z* 183.1→124.1 for veratric acid; the monitored ion transitions of the ILV were similar to those of the ECM.

3 Veratric acid recovery results are reported based on the re-analysis results of the samples. Original recovery results were 55.8-68.4% (Q) and 68.7-86.8% (C) for the LOQ analysis and 82.3-90.1% (Q) and 88.8-92.6% (C) for the 10×LOQ analysis (Tables 17-18, pp. 53-54).

III. Method Characteristics

The LOQ for veratridine and veratric acid in water was reported as 0.10 µg/L in the ECM and ILV (pp. 27-28, 32-33, 35-37 of MRID 51457864; p. 33 of MRID 51457865). In the ECM, the LOQ was defined as the lowest fortification level where blank values did not exceed 30% of the LOQ. No justifications for the LOQ were reported in the ILV. No calculations for the LOQ were reported in the ILV or ECM. The LOD in ground and surface water was calculated as 0.00834-0.0150 µg/L and 0.0141-0.0194 µg/L for veratridine and veratric acid, respectively, in the ECM. The LOD was calculated in the ECM using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for n -1 (3.143 for seven replicates) and SD is the standard deviation of the analyte recovery measurements at the estimated LOQ.

The LOD was not reported for veratridine and veratric acid in the ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Veratridine	Veratric acid
Limit of Quantitation (LOQ)*	ECM	0.0997 µg/L[1]	0.10 µg/L
	ILV	0.10 µg/L	
Limit of Detection (LOD)	ECM	0.00869-0.0147 µg/L (GW, calc) 0.00834-0.0150 µg/L (SW, calc)	0.0169-0.0194 µg/L (GW, calc) 0.0141-0.0154 µg/L (SW, calc)
	ILV	**Not reported**	
Linearity (calibration curve r and concentration range)	ECM[2]	r = 0.9995 (GW, Q) r = 0.9985 (GW, C) r = 0.9980 (SW, Q) r = 0.9975 (SW, C)	r = 0.9995 (GW, Q & C) r = 0.9990 (SW, Q) r = 0.9985 (SW, C)
		0.00498-0.0796 ng/L	0.250-4.00 ng/L
	ILV[3]	r = 0.9989 (GW, Q & C) r = 0.9994 (GW, Q & C) r = 0.9999 (SW, Q) r = 0.9996 (SW, C)	r = 0.9976 (GW, Q) r = 0.9977 (GW, C) r = 0.9976 (SW, Q) r = 0.9975 (SW, C)
		0.0050-0.0800 ng/L	0.0250-0.400 ng/L
Repeatable	ECM[4]	Yes for LOQ (0.0997 µg/L) and 10×LOQ (0.997 µg/L) in characterized surface and ground water matrices	Yes for LOQ (0.10 µg/L) and 10×LOQ (1.00 µg/L) in characterized surface and ground water matrices
	ILV[3,5,6]	Yes for LOQ (0.10 µg/L) and 10×LOQ (1.00 µg/L) in characterized surface and ground water matrices	
Reproducible[7]		Yes for 0.10 µg/L (LLMV)* and 1.00 µg/L in two water matrices.	
Specific	ECM	Yes, matrix interferences were <5% (based on peak area). Some peak tailing was observed.	Yes, matrix interferences were <5% (based on peak area). Baseline was elevated around analyte peak.
	ILV[3]	Yes, matrix interferences were <7% of the LOQ (based on peak height).	Yes, matrix interferences were <7% (Q) and 25% (C) of the LOQ (based on peak height). Baseline was elevated around analyte peak. Baseline noise interfered with peak integration and attenuation at the LOQ.

Data were obtained from pp. 27-28, 32-33, 35-37 (LOQ/LOD); p. 34 (linearity coefficients & data); Tables 1-8, pp. 41-48 (recovery data); Figures 49-56, pp. 109-116 (calibration curves); Figures 1-40, pp. 61-100 (chromatograms) of MRID 51457864; p. 33 (LOQ/LOD); p. 26 (linearity coefficients); Tables 9-20, pp. 45-56 (recovery data); p. 19; Appendix E, pp. 94-107 (linearity coefficients); Appendix F, Figures 33-77, pp. 146-190 (chromatograms & calibration curves) of MRID 51457865; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition; SW = Surface water; GW = Ground water.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 The reported ILV LOQ for veratridine differed slightly from the reported ECM LOQ for veratridine (p. 33 of MRID 51457864; pp. 33 of MRID 51457865). The difference was due to an ECM adjustment for test material purity (pp. 15, 19, 22-23 of MRID 51457864; see Reviewer's Comment #1).

2 Reported r values were reviewer-calculated from r^2 values reported in the study report (p. 34 of MRID 51457864; DER Attachment 2). Values were reported to four significant figures.

3 Values for veratric acid analysis were taken from the re-analysis (10-fold dilution of the original samples).

4 In the ECM, ground (well) water (SMV Nos. R04-20-18-17 & R04-20-18-18; collected on April 18, 2018; pH 8.0, hardness 192 mg/L $CaCO_3$, conductivity 0.47 mmhos/cm, total dissolved solids 220 ppm), which was obtained from Research For Hire, Porterville, California, and surface water (SMV No. R03-06-18-07; collected on February 27, 2018; pH 8.0, hardness 130 mg/L $CaCO_3$, conductivity 0.37 mmhos/cm, total dissolved solids 174 ppm) which was obtained from Turner Ag Research, Inc., Yuba City, California, were used in the study (pp. 17-18 of MRID 51457864). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

5 In the ILV, ground (well) water (Sample ID: WELLW-0612190B; collected on June 12, 2019; pH 7.9, hardness 209 mg/L $CaCO_3$, conductivity 0.49 mmhos/cm, total dissolved solids 356 ppm) and surface (irrigation canal) water (Sample ID: HERNDON-39-061219-B; collected on June 12, 2019; pH 6.9, hardness 12 mg/L $CaCO_3$, conductivity 0.07 mmhos/cm, total dissolved solids 22 ppm) were obtained from Fresno, California, and used in the study (pp. 22-23; Appendix D, pp. 81-82 of MRID 51457865). The ground water was filtered prior to use. Both water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

6 The method was validated by the ILV with the first trial for veratridine and veratric acid in both water matrices with minor modifications to the sample processing of the final extracts for veratric acid analysis (including the 10-fold dilution) and with insignificant modifications to the analytical parameters and equipment (pp. 15-26 of MRID 51457865). The extraction modification for veratric acid was made to the method due to low recoveries in the first injections of ground and surface water (p. 24). The low recoveries were assumed to be used to labware interference (p. 26). The ILV could not employ the glass centrifuge tubes which were advised by the ECM personnel, but the 10-fold extraction of the original extracts resulted in acceptable recoveries. Following the ILV suggestion, the ECM method should be "updated to specify that water samples should be extracted in glass" (p. 34). Additionally, the ECM method should be updated to advise the use of a 10-fold dilution for veratric acid analysis if glass laboratory extraction vessels cannot be used.

7 Since the ECM LOQ for veratridine was slightly less than the ILV LOQ for veratridine, the ILV LOQ and 10×LOQ for veratridine was considered to be supported by the ECM performance data at the ECM LOQ and 10×LOQ. for veratridine.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV author suggested that the ECM method should be "updated to specify that water samples should be extracted in glass" (p. 34 of MRID 51457865). The ILV employed extraction modifications (including the 10-fold dilution) for veratric acid analysis due to low recoveries in the first injections of ground and surface water (p. 24 of MRID 51457865). The low recoveries were assumed to be due to labware interference (p. 26). The ILV could not employ the glass centrifuge tubes which were advised by the ECM personnel, but the 10-fold extraction of the original extracts resulted in acceptable recoveries. Although the propensity of dichloromethane to dissolve plastics is known, the ECM method should also be updated to advise the use of a 10-fold dilution for veratric acid analysis if glass laboratory extraction vessels cannot be used. (p. 34)

   The reviewer noted that the propensity of dichloromethane to dissolve plastics is known in chemical laboratory practice literature.

2. In the the ECM, the storage stability of the veratridine and veratric acid in ground and surface water was determined to be up to 28 days under refrigerated conditions, except for veratric acid in surface water which was determined to be up to 7 days under refrigerated conditions (p. 35; Tables 13-20, pp. 53-60 MRID 51457864). Additionally, QC samples yielded acceptable (ranging 83.6 to 117%) for veratridine and veratric acid in soil and sediment at the nominal fortified concentrations.

3. The ECM Protocol stated that the LOQ and 10×LOQ validation fortifications were to be 0.100 µg/L and 1.0 µg/L, respectively (Appendix 1, p. 121 of MRID 51457864). However, the ECM validated the water method for veratridine at LOQ and 10×LOQ validation fortifications of 0.0997 µg/L and 0.997 µg/L, respectively, instead of 0.100 µg/L and 1.0 µg/L, respectively, due to ECM adjustment for test material purity (pp. 15, 19-20, 22-23). The veratridine test material purity was 94.1% (p. 15). In order to prepare the stock solutions for the LOQ and 10×LOQ validation fortifications, 0.0099 g was weighed out which, based on the known purity, was equivalent to 0.0093 g a.i. yielding a 932 mg a.i./L primary stock solution concentration and 99.7 µg/L and 9.97 µg/L sub-stock solution concentrations (pp. 19-20). With the 0.200 mL application into 20.0 mL total volume, the veratridine LOQ and 10×LOQ validation fortifications were 0.0997 µg/L and 0.997 µg/L, respectively (pp. 22-23). This ECM adjustment for test material purity caused the ECM LOQ and 10×LOQ fortifications to differ slightly from the ILV LOQ and 10×LOQ fortifications.

   The reviewer noted that the same veratridine test material (purity 94.1%) was used in the ILV; however; 0.0110 g of this test material was weighed out to prepare the stock solutions for fortifications (pp. 16-17 of MRID 51457865).

4. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 27-28, 32-33, 35-37 of MRID 51457864; p. 33 of MRID 51457865). In the ECM, the LOQ was defined as the lowest fortification level where blank values did not exceed 30% of the LOQ. No justifications for the LOQ were reported in the ILV. No calculations for the LOQ were reported in the ILV or ECM. The LOD was calculated in the ECM using the following equation: LOD = (SD × $t_{0.99}$), where $t_{0.99}$ is the one-tailed t statistic for n -1 (3.143 for seven replicates) and SD is the standard deviation of the analyte recovery measurements at the estimated LOQ. The LOD was not reported for veratridine and veratric acid in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ILV reported method LOQ for both analytes in water (0.100 µg/L).

The reviewer noted that the LOD equation was equivalent to the method detection limit (MDL) as defined in the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

5. The communication between the ILV Study Director/Author (Elisabeth Schoenau, Golden Pacific Laboratories, LLC) and Study Monitor (Mark Lenz, Exponent®) was summarized and listed but not detailed (pp. 1, 33-35; Appendix A, p. 59 of MRID 51457865). Communications involved protocol issue, ECM method report issue, and informing the Study Monitor of the success/results of ILV trial. The only method specific communication which was exchanged between the ECM laboratory personnel and ILV laboratory personnel, which was not specified in Smithers Viscient Analytical Method 14012.6138, was the "size and container type" for the sample aliquot measurement (p. 34). The discussion between the Study Director and Study Monitor regarding the dichloromethane/glass concern appeared to involve miscommunication and discord over the reason for the ILV validation issues (pp. 34-35).

6. In the ECM, no significant matrix effects were observed (<20%; pp. 33, 37-38; Tables 9-12, pp. 49-52 of MRID 51457864). No matrix effect studies were conducted in the ILV.

7. The time requirement for the method was reported in the ILV as *ca*. 4-5 hours for sample preparation and processing, *ca*. 3-4 hours for LC/MS/MS analysis, and 2 hours for calculations (the reference to "soil or sediment" was assumed to be typographical error; p. 27 of MRID 51457865). The total time for a sample set was reported as up to two calendar days. In the ECM, the time requirement for the method was reported as *ca*. 7-8 hours for sample preparation and processing, LC/MS/MS analysis overnight, and 2 hours for calculations (p. 34 of MRID 51457864).

V. References

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

U.S. Environmental Protection Agency (USEPA). 2012a. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

USEPA. 2012b. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

Urann, K. 2018. Saba10-Early Life-Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*). Unpublished study performed by Smithers Viscient, Wareham, Massachusetts. Laboratory Study No. 14012.6153. Study sponsored by MGK®, Minneaplois, Minnesota. MRID 51457881/50609501.

Attachment 1: Chemical Names and Structures

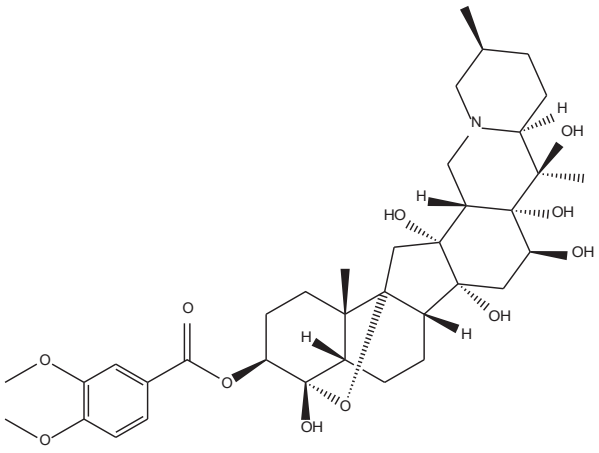
Veratridine (component of Sabadilla Alkaloids; 3-Veratroylveracevine)

IUPAC Name: (3S,4S,4aS,6aS,6bR,8S,8aS,9R,9aS,12S,15aS,15bR,16aR,16bS)-4,6b,8,8a,9,15b-hexahydroxy-9,12,16b-trimethyldocosahydro-2H-4,16a-epoxybenzo[4,5]indeno[1,2-h]pyrido[1,2-b]isoquinolin-3-yl 3,4-dimethoxybenzoate

CAS Name: Not reported

CAS Number: 71-62-5

SMILES String: COC1=C(OC)C=CC(C(O[C@@H]2[C@@](O)(O3)[C@](CC[C@]4([H])[C@]53C[C@]6(O)[C@@]4(O)C[C@H](O)[C@@]7(O)[C@@]6([H])CN8[C@](CC[C@H](C)C8)([H])[C@]7(O)C)([H])[C@]5(C)CC2)=O)=C1



Veratric acid

IUPAC Name: 3,4-Dimethoxybenzoic acid
CAS Name: Not reported
CAS Number: 93-07-2
SMILES String: O=C(O)C1=CC(OC)=C(OC)C=C1