
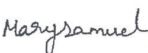


Analytical method for Veratridine, a component of *Sabadilla* Alkaloids, and its major degradate Veratric acid in soil and sediment

- Reports:** ECM: EPA MRID No. 51457863. Jutson, J.I. 2018. Validation of the Analytical Method for the Determination of Veratridine and its Major Metabolite in Soil and Sediment by LC-MS/MS. Smithers Viscient Study No.: 14012.6139. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by MGK[®] Minneapolis, Minnesota; 130 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 51457863 & 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 51457863). 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 **Supplemental**. The LOQ is greater than the lowest toxicological level of concern. Also, the LOD was not reported in the ILV.

PC Code:	002203 (SABA-10); PC 002201 (Sabadilla Alkaloids)		
Final EPA Reviewer:	A'ja V. Duncan, Ph.D. Chemist	Signature: Date:6/22/22	AJA DUNCAN <small>Digitally signed by AJA DUNCAN Date: 2022.06.22 13:02:02 -04'00'</small>
	Ibrahim Abdel-Saheb, Ph.D., Environmental Scientist	Signature: Date:6/22/22	IBRAHIM ABDEL SAHEB <small>Digitally signed by IBRAHIM ABDEL SAHEB Date: 2022.06.22 09:33:30 -04'00'</small>
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	 10/26/2021
	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	 10/26/2021

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 14012.6139, is designed for the quantitative determination of veratridine and its major degradate veratric acid at 49.9 µg/kg and 50.0 µg/kg, respectively, in soil and sediment using LC/MS/MS. The LOQs are greater than the lowest toxicological level of concern (12 µg/kg, for dicots; MRID 50561402) in soil and sediment for both analytes.

The reported ILV LOQ for veratridine (50.0 µg/kg) differed slightly from the reported ECM LOQ for veratridine due to the ECM adjustment for test material purity. 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. 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 soil and sediment (50.0 µg/kg).

The ECM and ILV validated the method using the same characterized sandy loam soil and clay sediment matrices; however, the soil and sediment characterization data was not reproduced in the ILV study report. It could not be determined if the ILV soil and sediment matrices covered the range of soils used in the TFD studies since no veratridine TFD studies were submitted. The ILV validated the method with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications. 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	51457863 ¹	51457865 ²		Soil and Sediment	28/11/2018	McLaughlin Gormley King Co. (MGK)	LC/MS/MS	50.0 µg/kg (ILV) ³
Veratric acid								50.0 µg/kg

1 In the ECM, the matrices were sandy loam soil (Smithers Viscient Batch No.: R03-14-18-09; pH 8.6 (1:1 soil:water ratio); 73% sand, 20% silt, 7% clay, 0.63% organic matter) and clay sediment (Smithers Viscient Batch No.: R03-06-18-08; pH 7.4 (1:1 sediment:water ratio); 25% sand, 34% silt, 41% clay, 2.1% organic matter); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification p. 16 of MRID 51457863). The soil and sediment textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.

2 In the ILV, the soil and sediment matrices were the same as those used in the ECM; however, the soil and sediment characterization data was not reproduced in the ILV study report (p. 21 of MRID 51457865).

3 The reported ILV LOQ for veratridine differed slightly from the reported ECM LOQ for veratridine (p. 31 of MRID 51457863; pp. 33 of MRID 51457865). The difference was due to an ECM adjustment for test material purity (pp. 14-15, 18, 21 of MRID 51457863; see Reviewer's Comment #1).

I. Principle of the Method

Samples (5.00 g, dry weight) of soil and sediment were fortified with 250 µL of 0.997 mg/L or 9.97 mg/L veratridine fortification solutions or 250 µL of 1.00 mg/L or 10.0 mg/L veratric acid fortification solutions, as necessary, then extracted twice with 20 mL of acetonitrile:0.1N hydrochloric acid solution (5:1, v:v) for 30 minutes on a shaker table (200 rpm) then centrifuged (3000 rpm for 10 minutes; pp. 20-23 of MRID 51457863). The volume of the combined extracts was adjusted to 50 mL with acetonitrile:0.1N hydrochloric acid solution (5:1, v:v). The recovery samples were further diluted (500xs/50xs for LOQ samples and 5000xs/500xs for 10×LOQ samples – veratridine/veratric acid) into the calibration range with acetonitrile:purified reagent water (10:90, v:v). Soil samples were centrifuged (13,000 rpm for five minutes) prior to LC/MS/MS analysis, while sediment samples were directly analyzed by HPLC.

Samples were analyzed for veratridine and veratric acid using a Shimadzu LC-20AD HPLC coupled to an MDS Sciex API 5000 and API 6500+ Qtrap mass spectrometer equipped with an ESI Turbo V interface in the positive ion, multiple reaction monitoring (MRM) mode (pp. 16, 24-25 of MRID 51457863). The following LC conditions were used: Waters XBRidge 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. 0.0:100.0, 4.10-6.00 min. 90.0:10.0], MS temperature 600°C, and injection volume of 5.0-20.0 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; however, a reduced injection volume (5.0 µL) was used for analysis of soil extracts to minimize matrix enhancement (p. 31).

The ILV performed the ECM method, Smithers Viscient Analytical Method 14012.6139, as written, except for the fact that the samples were diluted 50xs/5xs for veratridine/veratric acid prior to analysis and for insignificant modifications to the analytical parameters and equipment (pp. 15-22, 24-26; Appendix C, p. 77 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 μ L. 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 \rightarrow 456.4 and m/z 674.4 \rightarrow 165.1 for veratridine, and m/z 183.1 \rightarrow 139.1 and m/z 183.1 \rightarrow 124.1 for veratric acid; the monitored ion transitions of the ILV were similar to those of the ECM. The ILV modifications do not require an updated ECM method.

The Limit of Quantification (LOQ) in soil and sediment was reported as 49.9 μ g/kg and 50.0 μ g/kg for veratridine and veratric acid, respectively, in the ECM and 50.0 μ g/kg for veratridine and veratric acid in the ILV (pp. 26, 30-31, 35-37 of MRID 51457863; p. 33 of MRID 51457865). The Limit of Detection (LOD) in soil and sediment was calculated as 4.3-6.3 μ g/kg and 1.4-7.1 μ g/kg 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 51457863): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of veratridine in one soil matrix and one sediment matrix at the LOQ (49.9 μ g/kg) and 10 \times LOQ (499 μ g/kg; Tables 1-8, pp. 40-47). Mean recoveries and RSDs met requirements for analysis of veratric acid in one soil matrix and one sediment matrix at the LOQ (50.0 μ g/kg) and 10 \times LOQ (500 μ g/kg). Recovery results of the quantitative and confirmatory ion transitions were comparable, except for the LOQ analyses of both analytes in sediment where recovery results of the confirmatory ion transition were significantly less than those of the respective quantitative ion transition. The matrices were sandy loam soil (Smithers Viscient Batch No.: R03-14-18-09; pH 8.6 (1:1 soil:water ratio); 73% sand, 20% silt, 7% clay, 0.63% organic matter) and clay sediment (Smithers Viscient Batch No.: R03-06-18-08; pH 7.4 (1:1 sediment:water ratio); 25% sand, 34% silt, 41% clay, 2.1% organic matter); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification p. 16). Soil sources were not reported.

ILV (MRID 51457865): Mean recoveries and RSDs met requirements for analysis of veratridine and veratric acid in two soil and sediment matrices at the LOQ (50.0 μ g/kg) and 10 \times LOQ (500 μ g/kg; Tables 1-8, pp. 37-44). Recovery results of the quantitative and confirmatory ion transitions were comparable. The soil and sediment matrices were the same as those used in the ECM; however, the soil and sediment characterization data was not reproduced in the ILV study report (p. 21). The method, Smithers Viscient Analytical Method 14012.6139, was validated by

the ILV with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications (pp. 15-22, 24-26, 34-35). The LOQ and 10×LOQ fortification concentrations of veratridine differed slightly from those of the ECM, due to an ECM adjustment for test material purity (pp. 14-15, 18, 21 of MRID 51457863; see Reviewer's Comment #1).

Table 2. Initial Validation Method Recoveries for Veratridine and Veratric acid in Soil and Sediment¹

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Veratridine	49.9 (LOQ)	7	101-112	106	4.20	3.94
	499	5	101-112	106	4.82	4.54
Veratric acid	50.0 (LOQ)	7	86.1-95.4	90.8	3.43	3.78
	500	5	90.5-101	98.1	4.36	4.44
Confirmation ion transition						
Veratridine	49.9 (LOQ)	7	107-114	110	2.63	2.40
	499	5	100-109	106	3.77	3.56
Veratric acid	50.0 (LOQ)	7	88.2-90.5	89.6	0.910	1.02
	500	5	91.7-105	97.7	4.98	5.10
Clay Sediment						
Quantitation ion transition						
Veratridine	49.9 (LOQ)	7	108-115	112	3.34	2.98
	499	5	99.6-113	104	5.79	5.56
Veratric acid	50.0 (LOQ)	7	109-118	114	3.25	2.86
	500	5	99.7-113	105	5.78	5.49
Confirmation ion transition						
Veratridine	49.9 (LOQ)	7	92.2-102	97.2	3.48	3.59
	499	5	102-105	103	1.34	1.30
Veratric acid	50.0 (LOQ)	7	90.3-103	97.0	4.52	4.66
	500	5	100-109	104	3.35	3.22

Data (uncorrected results, pp. 29-30) were obtained from Tables 1-8, pp. 40-47 of MRID 51457863. 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 matrices were sandy loam soil (Smithers Visient Batch No.: R03-14-18-09; pH 8.6 (1:1 soil:water ratio); 73% sand, 20% silt, 7% clay, 0.63% organic matter) and clay sediment (Smithers Visient Batch No.: R03-06-18-08; pH 7.4 (1:1 sediment:water ratio); 25% sand, 34% silt, 41% clay, 2.1% organic matter); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification p. 16). The soil and sediment textures were verified by the reviewer using USDA-NRCS technical support tools.

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 Soil and Sediment¹

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Veratridine	50.0 (LOQ)	5	104-106	105	0.894	0.851
	500	5	98.8-102	101	1.40	1.39
Veratric acid	50.0 (LOQ)	5	99.6-102	101	0.976	0.966
	500	5	99.2-108	104	3.29	3.16
Confirmation ion transition						
Veratridine	50.0 (LOQ)	5	102-106	104	1.48	1.42
	500	5	98.0-103	101	2.16	2.14
Veratric acid	50.0 (LOQ)	5	99.4-102	101	1.31	1.30
	500	5	100-108	104	2.92	2.81
Clay Sediment						
Quantitation ion transition						
Veratridine	50.0 (LOQ)	5	96.8-102	100	2.05	2.05
	500	5	95.8-103	101	2.96	2.93
Veratric acid	50.0 (LOQ)	5	100-103	102	1.52	1.49
	500	5	102-115	108	4.74	4.39
Confirmation ion transition						
Veratridine	50.0 (LOQ)	5	96.8-102	100	2.01	2.01
	500	5	95.0-103	99.8	3.36	3.37
Veratric acid	50.0 (LOQ)	5	96.2-103	101	2.78	2.75
	500	5	106-116	110	3.90	3.55

Data (uncorrected results, pp. 27-29) were obtained from Tables 1-8, pp. 37-44 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 soil and sediment matrices were the same as those used in the ECM; however, the soil and sediment characterization data was not reproduced in the ILV study report (p. 21).
- 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.

III. Method Characteristics

The LOQ in soil and sediment was reported as 49.9 µg/kg and 50.0 µg/kg for veratridine and veratric acid, respectively, in the ECM and 50.0 µg/kg for veratridine and veratric acid in the ILV (pp. 26, 30-31, 35-37 of MRID 51457863; 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 soil and sediment was calculated as 4.3-6.3 µg/kg and 1.4-7.1 µg/kg for veratridine and veratric acid, respectively, in the ECM. The LOD was calculated in the ECM using the following equation:

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

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	49.9 µg/kg ¹	50.0 µg/kg
	ILV	50.0 µg/kg	
Limit of Detection (LOD)	ECM	4.3-6.3 µg/kg (Soil, calc) 5.2 µg/kg (Sed, calc)	1.4-5.4 µg/kg (Soil, calc) 5.5-7.1 µg/kg (Sed, calc)
	ILV	Not reported	
Linearity (calibration curve r and concentration range)	ECM ²	r = 0.9965 (Soil, Q) r = 0.9975 (Soil, C) r = 0.9985 (Sed, Q) r = 0.9990 (Sed, C)	r = 0.9990 (Soil, Q & C) r = 0.9995 (Sed, Q) r = 0.9990 (Sed, C)
		0.0250-0.500 µg/L	0.250-5.00 µg/L
	ILV	r = 0.9999 (Soil, Q & C) r = 0.9999 (Sed, Q & C)	r = 0.9996 (Soil, Q) r = 0.9994 (Soil, C) r = 0.9993 (Sed, Q) r = 0.9996 (Sed, C)
		0.0253-0.505 µg/L	0.250-5.00 µg/L
Repeatable	ECM ³	Yes for LOQ (49.9 µg/kg) and 10×LOQ (499 µg/kg) in characterized soil and sediment matrices	Yes for LOQ (50.0 µg/kg) and 10×LOQ (500 µg/kg) in characterized soil and sediment matrices
	ILV ^{4,5}	Yes for LOQ (50.0 µg/kg) and 10×LOQ (500 µg/kg) in characterized soil and sediment matrices	
Reproducible ⁶		Yes for 50.0 µg/kg (LLMV)* and 500 µg/kg in soil and sediment matrices	
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak area). Some baseline noise was observed near the analyte peak which interfered with peak integration and attenuation.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline was elevated around analyte peak.
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak height).	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline was elevated around analyte peak.

Data were obtained from pp. 26, 30-31, 35-37 (LOQ/LOD); pp. 31-32 (linearity coefficients & data); Tables 1-8, pp. 40-47 (recovery data); Figures 49-56, pp. 108-115 (calibration curves); Figures 1-40, pp. 60-99 (chromatograms) of MRID 51457863; p. 33 (LOQ/LOD); Tables 1-8, pp. 37-44 (recovery data); p. 18; Appendix E, pp. 85-92 (linearity coefficients); Appendix F, Figures 1-32, pp. 114-145 (chromatograms & calibration curves) of MRID 51457865; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition; Soil = Sandy loam soil; Sed = Clay sediment.

* 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. 31 of MRID 51457863; pp. 33 of MRID 51457865). The difference was due to an ECM adjustment for test material purity (pp. 14-15, 18, 21 of MRID 51457863; see Reviewer's Comment #1).
- 2 Reported r values were reviewer-calculated from r² values reported in the study report (p. 26 of MRID 51457863; DER Attachment 2). Values were reported to four significant figures.
- 3 In the ECM, the matrices were sandy loam soil (Smithers Viscient Batch No.: R03-14-18-09; pH 8.6 (1:1 soil:water ratio); 73% sand, 20% silt, 7% clay, 0.63% organic matter) and clay sediment (Smithers Viscient Batch No.: R03-06-18-08; pH 7.4 (1:1 sediment:water ratio); 25% sand, 34% silt, 41% clay, 2.1% organic matter); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification p.

- 16 of MRID 51457863). The soil and sediment textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.
- 4 In the ILV, the soil and sediment matrices were the same as those used in the ECM; however, the soil and sediment characterization data was not reproduced in the ILV study report (p. 21 of MRID 51457865).
 - 5 The ILV validated the method for both analytes in the tested soil and sediments with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications (15-22, 24-26, 34-35 of MRID 51457865).
 - 6 Since the ECM LOQ for veratridine was slightly less than the ILV LOQ for veratridine, the ILV LOQ and $10\times$ LOQ for veratridine was considered to be supported by the ECM performance data at the ECM LOQ and $10\times$ LOQ for veratridine.

IV. Method Deficiencies and Reviewer's Comments

1. The LOQs are greater than the lowest toxicological level of concern (12 $\mu\text{g}/\text{kg}$, for dicots; MRID 50561402) in soil and sediment for both analytes.
2. 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 14012.6139, was the "size and container type" for the sample aliquot measurement (p. 34).
3. In the ECM, the storage stability of the veratridine and veratric acid in soil and sediment was determined to be up to 28 days under refrigerated conditions during the 28-day study duration (pp. 32-34; Tables 13-20, pp. 52-59 MRID 51457863). Additionally, QC samples yielded acceptable recoveries (ranging 80.1 to 115%) for veratridine and veratric acid in soil and sediment at the nominal fortified concentrations.
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. 26, 30-31, 35-37 of MRID 51457863; 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: $\text{LOD} = (\text{SD} \times t_{0.99})$, 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. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the reported method 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 (pp. 26, 30-31, 35-37 of MRID 51457863; pp. 33 of MRID 51457865). 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 soil and sediment (50.0 µg/kg).

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. It could not be determined if the ILV soil and sediment matrices covered the range of soils used in the terrestrial field dissipation (TFD) studies since no veratridine terrestrial field dissipation studies were submitted. In the submitted veratridine aerobic soil metabolism study (MRID 51457857), four soils were studied: one each of sand, loam, sandy clay loam, and sandy loam soils (p. 16 of MRID 51457857). In the submitted veratridine aerobic aquatic metabolism study (MRID 51457859) and anaerobic aquatic metabolism study (MRID 51457860), two sediment-water systems were studied: silt loam and sand sediments (p. 17 of MRID 51457859; p. 17 of MRID 51457860).

The reviewer noted that the ILV soil and sediment matrices were the same as those of the ECM; however, the soil and sediment characterization data was not reproduced in the ILV study report (p. 21 of MRID 51457865).

6. The ECM Protocol stated that the LOQ and 10×LOQ validation fortifications were to be 50.0 µg/kg and 500 µg/kg, respectively (Appendix 1, p. 120 of MRID 51457863). However, the ECM validated the soil and sediment method for veratridine at LOQ and 10×LOQ validation fortifications of 49.9 µg/kg and 499 µg/kg, respectively, instead of 50.0 µg/kg and 500 µg/kg, respectively, due to ECM adjustment for test material purity (pp. 14-15, 18, 21). The veratridine test material purity was 94.1% (pp. 14-15). 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 0.997 mg/L and 9.97 mg/L sub-stock solution concentrations (p. 18). With the 0.250 mL application, the veratridine LOQ and 10×LOQ validation fortifications were 49.9 µg/kg and 499 µg/kg, respectively (p. 21). 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).

7. In the ECM, no significant matrix effects were observed (<20%; pp. 31, 35-37; Tables 9-12, pp. 48-51 of MRID 51457863). However, due to matrix effect observed for soil during analysis, a reduced injection volume (5.0 μ L) was used for analysis to minimize matrix enhancement. No matrix effect studies were conducted in the ILV.
8. The time requirement for the method was reported in the ILV as *ca.* 4 hours for sample preparation and processing, *ca.* 3-4 hours for LC/MS/MS analysis, and 2 hours for calculations (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. 32 of MRID 51457863).

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>.
- Marchessault, N.S. 2018. Sabadilla Alkaloids- Seedling Emergence Test. Unpublished study performed by Smithers Viscient, Warham, Massachusetts, and sponsored by MGK[®], Minneapolis, Minnesota. Laboratory Study Number 14012.6156. MRID 50561402.

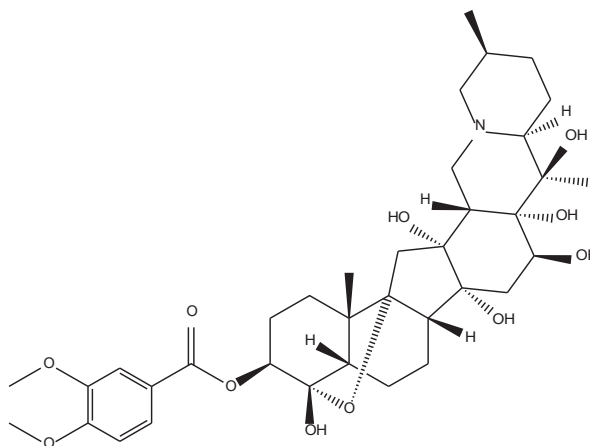
Attachment 1: Chemical Names and Structures**Veratridine (component of Sabadilla Alkaloids; 3-Veratroylveracevine)**

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