

Analytical method for strychnine in soil

Reports: ECM: EPA MRID No.: 50575601. Tsui, G. 2018. Method Validation - Analytical Method for the Determination of Residues of Strychnine in Soil. Study No. PB/18/001. Report prepared by Battelle UK Ltd., Essex, United Kingdom, and sponsored and submitted by H. Interdonati, Inc., Cold Spring Harbor, New York; 40 pages. Final report issued February 1, 2018.

ILV: EPA MRID No. 50557701. Thorn, J. 2018. Independent Laboratory Validation for the Determination of Residues of Strychnine in Soil by LC-MS/MS. Battelle Report/Study No. 100111019. Report prepared by Battelle, Norwell, Massachusetts, and sponsored and submitted by H. Interdonati, Inc., Cold Spring Harbor, New York; 34 pages. Final report with amendments issued March 27, 2018.

Document No.: MRIDs 50575601 & 50557701

Guideline: 850.6100


Statements: ECM: The study was conducted in accordance with OECD and UK Good Laboratory Practice (GLP) standards, as accepted by Regulatory Authorities throughout the European Community, the United States of America and Japan (p. 3; Appendix 4, p. 40 of MRID 50575601). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-4; Appendix 4, p. 40).

ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160) standards (p. 3 of MRID 50557701). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-4). Itemized changes to the report in Amendments 1 and 2 were reported (p. 1A).

Classification: This analytical method is classified as Acceptable. No ECM or ILV performance data was submitted for the 10×LOQ fortification level, but acceptable results were obtained from the 10×LOQ fortification level.

PC Code: 076901

EFED Final Reviewer: Jessica L. O. Joyce, M.S. Signature:
Lead Physical Scientist Date: 2/3/2022

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S., Signature: 
Environmental Scientist

Date: 01/28/2019

Mary Samuel, M.S., Signature: 
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Date: 01/28/2019

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

Executive Summary

The analytical method, Battelle UK Ltd. Study No. PB/18/001, is designed for the quantitative determination of strychnine in soil at the LOQ of 0.05 mg/kg using LC/MS/MS. The LOQ is likely greater than the lowest toxicological level of concern in soil; however, if properly buried in bait boxes, it is not expected to contaminate soil for ingestion by birds and mammals. The ECM and ILV validated the method using the same characterized sediment matrix. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM in the first trial since the method was performed as written with only minor LC/MS instrument modifications. All submitted ECM and ILV data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable at the LOQ and 100×LOQ; however, no ECM or ILV performance data was submitted for the 10×LOQ fortification level.

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						
Strychnine	50575601 ¹	50557701 ²	Acceptable	Soil	01/02/2018	H. Interdonati, Inc.	LC/MS/MS	0.05 mg/kg

1 In ECM, sandy clay loam soil [Sample ID: MSL-PF 0-6"; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 13; Appendix 2, p. 36 of MRID 50575601). Soil was characterized by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, sandy clay loam soil [Agvise Sample ID: MSL-PF 0-6"; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 12; Appendix 2, p. 30 of MRID 50557701). Soil was characterized by Agvise Laboratories, Northwood, North Dakota. This was the same soil matrix which was used in the ECM.

I. Principle of the Method

Soil samples (2 g) in 50-mL centrifuge tubes were fortified (0.10 mL of 1.0 or 100 µg/mL fortification solutions), as necessary (pp. 15-16; Figure 1, p. 26 of MRID 50575601). The samples were extracted with 10 mL of 2M sodium hydroxide (aq.) solution via shaking on a wrist action shaker for 20 minutes. An aliquot of 10 mL of 5% ethanol in ethyl acetate was added to the sample followed by 10 mL of hexane. The sample was shaken for another 20 minutes, followed by centrifugation at 3000 rpm for three minutes. The supernatant was transferred to a 40 mL glass vial and the soil was extracted once more with 10 mL of 2M sodium hydroxide (aq), 10 mL of 5% ethanol in ethyl acetate and 10 mL of hexane. After centrifugation, the combined extracts were evaporated to dryness under a stream of nitrogen at 40°C. The dried residues were reconstituted with 5 mL of 0.1% formic acid in methanol by sonication, followed by the addition of 5 mL of 10mM ammonium formate (aq. pH 4). The sample was mixed again by sonication and vortex. For 100×LOQ samples, an aliquot of 0.10 mL was diluted with 0.90 mL of control matrix. An aliquot of 0.10 mL of each control, LOQ and diluted 100×LOQ samples sample was transferred into an autosampler vial and diluted with 0.90 mL of 10mM ammonium formate (aq. pH 4) and analyzed by LC/MS/MS analysis.

Strychnine was identified and quantified by LC/MS/MS using an Agilent 1200 HPLC coupled with an API 6500 MS with a Q Jet Ion Guide (pp. 16-18 of MRID 50575601). The following conditions were employed: Zorbax Eclipse XDB-C18 analytical column (150 mm x 4.6 mm, 5 µm particle size; column temperature 30 ± 1°C) eluted with a gradient mobile phase of (A) 10mM ammonium formate (aq.) adjusted to pH 4 with formic acid and (B) 0.1% formic acid in methanol [time, percent A:B; time 0-1.0 min. 100:0, 2.0-4.0 min. 0:100, 5.0-6.0 min. 100:0] using an injection volume of 10 µL and positive ion turbo spray ionization MRM scan mode (MS temperature 500°C). Strychnine was identified using two ion transitions (quantitation and confirmation, respectively): m/z 335→156 and m/z 335→184. Expected retention time was *ca.* 3.3 ± 0.5 minutes for strychnine.

In the ILV, the ECM was performed as written, except for insignificant LC/MS instrument modifications (pp. 13-15, 17; Figure 1, p. 20; Appendix 3, p. 31 of MRID 50557701). Analytes were identified and quantified by LC/MS/MS using a Nexera XR UHPLC coupled with a Sciex API 6500+ Triple Quad MS. The LC/MS conditions were the same as the ECM; the MS transitions were the same as those of ECM. Expected retention time was *ca.* 3.2 minutes for strychnine.

The Limit of Quantification (LOQ) and Limit of Determination (LOD) for strychnine in soil were 0.05 mg/kg and 0.0075 mg/kg, respectively, in the ECM and ILV (pp. 12, 20 of MRID 50575601; pp. 10, 16 of MRID 50557701).

II. Recovery Findings

ECM (MRID 50575601): Mean recoveries and RSDs were within guideline requirements [means between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$] for analysis of strychnine at fortification levels of 0.05 mg/kg (LOQ) and 5.00 mg/kg (100 \times LOQ) in one soil matrix (p. 12; Tables 3-4, pp. 23-24). No samples were prepared at the 10 \times LOQ fortification level. Strychnine was identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. Sandy clay loam soil [Sample ID: MSL-PF 0-6"; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 13; Appendix 2, p. 36). Soil was characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50557701): Mean recoveries and RSDs were within guideline requirements for analysis of strychnine at fortification levels of 0.05 mg/kg (LOQ) and 5.00 mg/kg (100 \times LOQ) in one soil matrix (p. 12; Table 3, p. 19; DER Attachment 2). No samples were prepared at the 10 \times LOQ fortification level. Strychnine was identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. Sandy clay loam soil [Agvise Sample ID: MSL-PF 0-6"; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 12; Appendix 2, p. 30). Soil was characterized by Agvise Laboratories, Northwood, North Dakota. This was the same soil matrix which was used in the ECM. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM in the first trial since the method was performed as written with only minor LC/MS instrument modifications (pp. 10, 17).

Table 2. Initial Validation Method Recoveries for Strychnine in Soil (ECM) ^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Clay Loam Soil						
Quantitation Ion Transition						
Strychnine	0.05 (LOQ)	6	97-106	102	3.1	3.1
	5.00	6	99-106	102	2.7	2.6
Confirmation Ion Transition						
Strychnine	0.05 (LOQ)	6	95-104	101	3.7	3.7
	5.00	6	101-108	104	2.6	2.5

Data (uncorrected recovery results, pp. 18-19) were obtained from p. 12; Tables 3-4, pp. 23-24 of MRID 50575601.

1 Sandy clay loam soil [Sample ID: MSL-PF 0-6²; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 13; Appendix 2, p. 36). Soil was characterized by Agvise Laboratories, Northwood, North Dakota.

2 Strychnine was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 335→156 and *m/z* 335→184.

Table 3. Independent Validation Method Recoveries for Strychnine in Soil (ILV) ^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Sandy Clay Loam Soil						
Quantitation Ion Transition						
Strychnine	0.05 (LOQ)	5	94-102	99	5	4.7
	5.00	5	90-106	96	6	6.5
Confirmation Ion Transition						
Strychnine	0.05 (LOQ)	5	92-97	95	5	2.3
	5.00	5	90-107	97	6	6.7

Data (uncorrected recovery results, pp. 15-16) were obtained from p. 12; Table 3, p. 19 of MRID 50557701 and DER Attachment 2.

1 Sandy clay loam soil [Agvise Sample ID: MSL-PF 0-6²; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 12; Appendix 2, p. 30). Soil was characterized by Agvise Laboratories, Northwood, North Dakota. This was the same soil matrix which was used in the ECM.

2 Strychnine was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 335→156 and *m/z* 335→184; the MS transitions were the same as those of the ECM.

3 Standard deviations were reviewer-calculated since these values were not reported in the study report. Rules of significant figures were followed.

III. Method Characteristics

The LOQ and LOD for strychnine in soil were 0.05 mg/kg and 0.0075 mg/kg, respectively, in the ECM and ILV (pp. 12, 20 of MRID 50575601; pp. 10, 16 of MRID 50557701). In the ECM and ILV, the LOD was based on the lowest calibration standard; no justifications were provided for LOQ. No calculations for the LOQ and LOD were provided in the ECM and ILV.

Table 4. Method Characteristics

Analyte		Strychnine
Limit of Quantitation (LOQ)		0.05 mg/kg
Limit of Detection (LOD)		0.0075 mg/kg
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9982$ (Q) $r^2 = 0.9986$ (C)
		0.150-12.0 ng/mL
	ILV	$r^2 = 0.9994$ (Q) $r^2 = 0.9996$ (C)
		0.150-12.05 ng/mL
Repeatable	ECM ³	Yes at LOQ and 100×LOQ (one characterized soil). No at 10×LOQ; no 10×LOQ samples prepared.
	ILV ^{4,5}	
Reproducible		Yes at LOQ and 100×LOQ. No at 10×LOQ.
Specificity		No 10×LOQ representative chromatograms were provided.
	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak area).
	ILV	Yes, matrix interferences were <10% of the LOQ (based on peak height).

Data were obtained from pp. 12, 20 (LOQ/LOD); p. 12; Tables 3-4, pp. 23-24 (recovery data); Figures 4-7, pp. 28-31 (chromatograms); Figures 8-9, pp. 32-33 (calibration curve) (chromatograms) of MRID 50575601; pp. 10, 16 (LOQ/LOD); p. 12; Table 3, p. 19 (recovery data); Figures 4-7, pp. 23-25 (chromatograms); Figures 8-9, pp. 26-27 (calibration curves); Figures 4-7, pp. 23-25 (chromatograms) of MRID 50557701; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 8-9, pp. 32-33 of MRID 50575601; Figures 8-9, pp. 26-27 of MRID 50557701; DER Attachment 2). Significant figures of r^2 were limited to four.

2 In ECM, sandy clay loam soil [Sample ID: MSL-PF 0-6²; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 13; Appendix 2, p. 36 of MRID 50575601). Soil was characterized by Agvise Laboratories, Northwood, North Dakota.

3 In the ILV, sandy clay loam soil [Agvise Sample ID: MSL-PF 0-6²; 65% sand, 12% silt, 23% clay; pH 6.8 (1:1 soil:water ratio), pH 6.5 (0.01M CaCl₂); 2.4% organic carbon] was used in the study (USDA Soil texture classification; p. 12; Appendix 2, p. 30 of MRID 50557701). Soil was characterized by Agvise Laboratories, Northwood, North Dakota. This was the same soil matrix which was used in the ECM.

4 The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM in the first trial since the method was performed as written with only minor LC/MS instrument modifications (pp. 10, 17 of MRID 50557701).

IV. Method Deficiencies and Reviewer's Comments

1. No ECM or ILV performance data was submitted for the 10×LOQ fortification level; therefore, the method was not validated at 10×LOQ. However, satisfactory linearity, specificity, accuracy and precision data were achieved at the LOQ and 100×LOQ, demonstrating that the method is suitable for quantification of residues of strychnine in soil.
2. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV soil matrix used in the study was the exact same as that used in the ECM (sandy clay loam soil - Sample ID: MSL-PF 0-6"; p. 13; Appendix 2, p. 36 of MRID 50575601; p. 12; Appendix 2, p. 30 of MRID 50557701). No strychnine terrestrial field dissipation studies were referenced or described.
3. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM in the first trial since the method was performed as written with only minor LC/MS instrument modifications (pp. 10, 17 of MRID 50557701).
4. Matrix effects were studied in the ECM and ILV and determined to be insignificant (p. 19; Table 2, p. 22 of MRID 50575601; pp. 16; Table 1, p. 18 of MRID 50557701). Solvent-based calibrations were used for analysis.
5. Storage stability was studied in the ECM, and it was determined that strychnine was stable in soil sample extracts for up to 3 days and in methanol for up to 74 days at a nominal temperature of 4°C (p. 19; Table 5, p. 25 of MRID 50575601). In the ILV, it was determined that strychnine was stable in soil sample extracts for up to 7 days and in methanol for up to 14 days at temperatures of <6°C but above freezing (p. 17; Table 2, p. 18 of MRID 50557701).
6. The ILV reported that no communication between the ILV and laboratories who developed and validated the methods other than to receive the method and study report (p. 17 of MRID 50557701). The reviewer noted that the performing laboratories of the ECM and ILV were both Battelle; however, the ECM was performed by Battelle UK Ltd., Essex, United Kingdom, and the ILV was performed by Battelle, Norwell, Massachusetts (pp. 1, 5 of MRID 50575601; pp. 1, 5 of MRID 50557701). The laboratory personnel differed between the two laboratories, as well.
7. For the ECM and ILV, the reviewer noted that matrix interferences in the quantitation ion chromatogram were less than those in the confirmation ion transition chromatogram (Figures 4-7, pp. 28-31 of MRID 50575601; Figures 4-7, pp. 22-25 of MRID 50557701).
8. The estimation of LOQ and LOD in the ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 12, 20 of MRID 50575601; pp. 10, 16 of MRID 50557701). In the ECM and ILV, the LOD was based on the lowest calibration standard; no justifications were provided for LOQ. No calculations for the

LOQ and LOD were provided in the ECM and ILV. Detection limits should not be based on arbitrary values.

9. In the ECM, the time required to complete the extraction of one set of 15 samples required *ca.* 9 hours of work, including calculation of results (p. 16 of MRID 50575601).
10. The list of insignificant modifications to the ILV study report were listed (p. 1A of MRID 50557701).

V. References

- U.S. Environmental Protection Agency. 2012. 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.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Strychnine****IUPAC Name:** Not reported**CAS Name:** Not reported**CAS Number:** 57-24-9**SMILES String:** O=C1N2c3c(C45C6N(CC4)CC4=CCOC(C(C4C6)C25)C1)cccc3