BATTELLE

50557701

STUDY TITLE

Independent Laboratory Validation for the Determination of Residues of Strychnine in Soil by LC-MS/MS

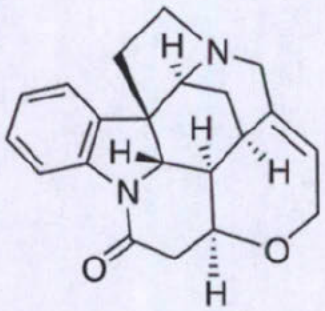
DATA REQUIREMENT

EPA Good Laboratory Practice Standards (40 CFR Part 160), EPA OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation, EU Guidance Documents SANCO/825/00 rev. 8.1, (16/11/2010) and SANCO/3029/99 rev. 4, (11/07/00)

2. OBJECTIVE

The objective of this study was to independently validate a previously reported method by Battelle UK Limited (BUKL) Study No. PB/18/001 "Method Validation – Analytical Method for the Determination of Residues of Strychnine in Soil" according to EPA Good Laboratory Practice Standards (40 CFR Part 160), EPA OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation, EU Guidance Documents SANCO/825/00 rev. 8.1, (16/11/2010) and SANCO/3029/99 rev. 4, (11/07/00).

3. TEST ITEM

Common Name:	Strychnine
CAS No.:	57-24-9
Molecular Formula:	$C_{21}H_{22}N_2O_2$
Molecular Weight:	334.41 g/mol
Molecular Structure:	
Supplier:	Sigma-Aldrich
Batch No.:	SZBE079XV
Purity:	99.9%
Expiry Date:	03/20/2019
Storage Conditions:	Ambient

4. TEST SYSTEMS

The samples used in this study was Agvise sample MSL-PF 0-6". The soil specimen will be stored in a freezer and will be supplied to the study with characterization details. Full details of the sources of the soil specimen and the characterization details are presented in **Appendix 2**.

All samples were stored in a freezer at ≤ -20°C when not in use.

5. METHOD VALIDATION

The determination of strychnine in soil matrices was performed using the method described in Battelle UK Limited Final Report for study number PB/18/001 (**Reference 1**).

The control samples were fortified as described in the following table:

Matrix	Untreated Control	Replicates at LOQ Fortification Level	Replicates at LOQ × 100 Fortification Level	Reagent Blank
Soil	2	5 at 0.05 mg/kg	5 at 5.0 mg/kg	1

LOQ = Limit of Quantification

The method was validated in terms of linearity, selectivity, accuracy and precision, monitoring two ion mass transitions for LC-MS/MS.

Matrix effects were investigated at the LOQ and LOQ×100 levels by comparing peak areas of solvent standard solutions to peak areas of matrix-matched standard solutions. Experiments assessed whether or not matrix effects were significant (i.e. >20% enhancement or suppression).

6. EXPERIMENTAL

6.1. Principle of the Method

Residues of strychnine were extracted from soil with 2M sodium hydroxide followed by partition with 5% ethanol in ethyl acetate and hexane. The extraction process was performed twice using a serial extraction approach. Extract were diluted in 10 mM ammonium formate adjusted to a pH of 4 with formic acid. The final determination by LC-MS/MS monitoring two ion mass transitions was carried out. The analytical flow chart is presented in **Figure 1**.

6.2. Equipment, Consumables, Reagents and Prepared Solutions

See **Appendix 3** for full details of all equipment, consumables, reagents and prepared solutions.

6.3. Standards and Fortifications

Stock solutions

Duplicate stock solutions, containing 1.0 mg/mL of strychnine, were prepared by dissolving 10 mg of the strychnine reference item in 10 mL of methanol. Separate stock solutions were prepared for the preparation of fortification solutions and the preparation of calibration solutions.

Fortification Solutions

Two fortification solutions, containing strychnine at 100 µg/mL and 1 µg/mL, were prepared by diluting appropriate amounts of one stock solution with methanol.

Fortifications

Matrix	Fortification Standard Concentration (µg/mL)	Fortification Volume (µL)	Weight of Sample (g)	Fortification Level (mg/kg)
Soil	1	100	2	0.05 (LOQ)
	100	100	2	5.0 (100 × LOQ)

Calibration

An intermediate solution, containing strychnine at 10 µg/mL, was prepared by diluting appropriate amounts of the second stock solution with methanol. This was further diluted with 10 mM ammonium formate at pH 4 to prepare 50 ng/mL and 5 ng/mL intermediate solutions.

Calibration solutions in 10 mM ammonium formate at pH 4 were prepared at concentrations of 0.15, 0.3, 1.0, 2.5, 5.0, 7.5, 10.0, and 12.0 ng/mL by diluting appropriate amounts of the 10, 1.0, and 0.1 µg/mL intermediate solutions. All standard solutions were stored at < 6°C but above freezing when not in use.

All samples were analyzed by liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS), monitoring two ion mass transitions.

Column: Agilent Zorbax Eclipse 5 µm XDB-C18 150x4.6 mm

Column Temperature: 30 °C

Injection Volume: 10 µL

Flow Rate: 1 mL/min

Mobile Phase A: 10 mM ammonium formate adjusted to pH 4 with formic acid

Mobile Phase B: 0.1 % formic acid in methanol

Time – minutes	% Mobile Phase A	% Mobile Phase B
0.00	100	0
1.00	100	0
2.00	0	100
4.00	0	100
5.00	100	0
6.00	100	0

Approximate Retention Time: Strychnine 3.2 minutes

Ion Source:	Turbo Ion Spray
Polarity:	Positive
Ion Spray Voltage (IS):	5500 v
Collision Gas (CAD):	Medium
Temperature (TEM):	500°C
Curtain Gas (CUR):	20
Ion Source Gas 1 (GS1):	60
Ion Source Gas 2 (GS2):	50
Entrance Potential (EP):	10

Analyte	Ion Mass Transitions (*m/z*)	Dwell Time (msec)	Declustering Potential (DP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)
Strychnine	335 → 156	150	110	63	10
	335 → 184	150	110	53	12

Valve Position:	Time (minutes)
B (waste)	0.0
A (detector)	2.0
B (waste)	4.5

7. CALIBRATION AND CALCULATIONS

Calibration curves were obtained from eight solvent-based calibration solutions containing strychnine, covering, at a minimum, a range of 0.15 ng/mL to 12.0 ng/mL (equivalent to 0.0075 mg/kg, the LOD, to 0.6 mg/kg). The curve was calculated by linear regression, with a weighting factor of 1/x. Correlation coefficients, r, greater than 0.995, demonstrating acceptable performance in terms of linearity.

The concentration of strychnine in the final sample extract was made by comparison to the calibration curve using the Analyst software.

The concentration in each sample was calculated as follows:

$$Stychnine\ \left(\frac{mg}{kg}\right) = \left(\frac{(P-c)*D*V*F}{m*W*A*1000}\right)$$

Where:

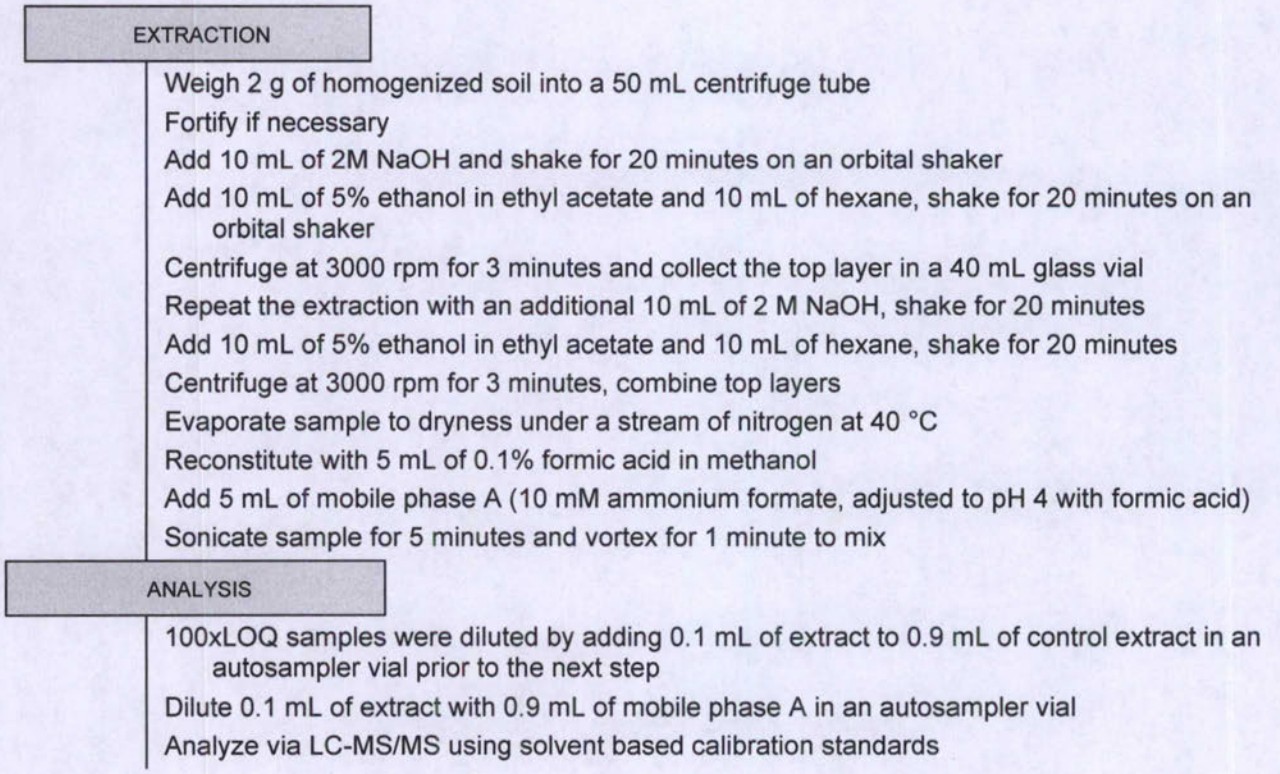
P = analyte peak area
M = slope of calibration curve
C = intercept of calibration curve
D = dilution factor (1 or 10)
V = extraction volume (10 mL)
F = final volume (1 mL)
W = weight (2 g)
A = aliquot volume (0.1 mL)

Procedural recovery data from fortified samples are calculated via the following equation:

Recovery = (Residue (mg/kg) ÷ Fortification (mg/kg)) × 100 %

14. FIGURES

Figure 1: Analytical Method Flow Chart for the Determination of Strychnine in Soil



Appendix 3: Equipment, Consumables, Reagents and Prepared Solutions

Equipment	**Manufacturer / Supplier**
Ultrasonic bath	Branson
N-Evap	Organomation Associates
Orbital Shaker Table	New Brunswick Scientific
Vortex mixer	Benchmark
Pipettes: Rainin Pipet Plus Rainin Pipet Lite	 Rainin Rainin
Balances: Mettler XS205 Mettler ML4002E/03	 Mettler Toledo Mettler Toledo
Centrifuges: Sorvall Legend XF	 Thermo Scientific
Millipore Water Purification System	Millipore
Glassware: Graduated cylinders Beakers Volumetric flasks	 Pyrex Pyrex Fisher Scientific
HPLC column: Zorbax Eclipse 5 µm XDB-C18 150x4.6 mm	 Agilent
Sciex API 6500+ Triple Quad Analyst 1.6.3 Software Nexera XR UHPLC System X2 SIL-30AC Autosampler	Sciex Shimadzu Shimadzu
IEC 1010 Model AGS400 NA dual bed nitrogen generator	Parker Hannifin Corporation
Consumables	
Glass vials (Crimp top) Glass Vials (40 mL)	Fisher Scientific Quality Environmental Containers
Polypropylene centrifuge tubes (50 mL)	Fisher Scientific
Crimp caps	Microliter
Caps (40 mL)	Quality Environmental Containers

Reagents	
Methanol (HPLC Grade)	Fisher Scientific
2-propanol / isopropyl alcohol (ACS grade)	Fisher Scientific
Hexane (Optima)	Fisher Scientific
Ethyl acetate (HPLC Grade)	Fisher Scientific
Ethanol (ACS Reagent)	Acros Organics
Sodium Hydroxide	JT Baker
Ammonium formate	Sigma Aldrich
Formic acid	Acros Organics
0.1% formic acid in methanol	Honeywell

Prepared Solutions
Mobile Phase A – 10 mM ammonium formate pH 4: 0.63 g of ammonium formate dissolved in Millipore water with 400 µL of formic acid added to adjust to pH 4.
Wash Solvent 1: dimethyl sulfoxide:isopropyl alcohol:acetonitrile:methanol (1:1:1:1 v/v/v/v)
Wash solvent 2: Water/Methanol (9:1 v/v)

Appendix 4: Abbreviations

Abbreviation	Definition
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CoA	certificate of analysis
conc.	concentration
Dil	dilution
DMSO	dimethyl sulfoxide
EC	European Commission
EPA	Environmental Protection Agency
ESP+	positive ion electrospray (mass spectrometry)
EU	European Union
GLP	Good Laboratory Practice(s)
HPLC	high performance liquid chromatography
i.d.	internal diameter
IUPAC	International Union of Pure and Applied Chemistry
LC-MS/MS	tandem liquid chromatograph-mass spectrometer-mass spectrometer
LOD	limit of detection
LOQ	limit of quantification
m/z	mass to charge ratio
MeCN	acetonitrile
MeOH	Methanol
mM	Millimolar
MS	mass spectrometry
n	number of data
NA	not applicable
NaOH	Sodium Hydroxide
ND	not detected
O.D.E	Oven dried equivalent
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organization for Economic Cooperation and Development
QAU	quality assurance unit
R (or r)	correlation coefficient
RSD	relative standard deviation
SD	standard deviation
STD	standard
UPW	ultra-pure water
v/v	volume for volume
Vf	final volume of the sample in mL
w/w	weight for weight