

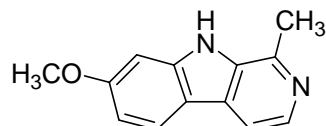


CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D884.2015.01

Compound Name: **Harmine**
Collection Number: D884
Chemical Formula: $C_{13}H_{12}N_2O$
CAS Number: 442-51-3
Structure:

Description: Off white needles
Batch Number: 04-D-21
Molecular Weight: 212.3
Release date: January 2005



Synonyms: 6-Methoxyharman; 7-Methoxy-1-methyl-9H-pyrido(3,4-b)indole,
1-Methyl-7-methoxy- β -carboline,
Telephathine

Purity (mass fraction): $99.9 \pm 0.3\%$ (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques, by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and 1H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

GC-FID: Instrument: Agilent 6890
Column: HP-1, 30 m x 0.32 mm I.D. x 0.25 μ m
Program: 180 $^{\circ}C$ (1 min), 10 $^{\circ}C/min$ to 300 $^{\circ}C$ (2 min)
Injector: 250 $^{\circ}C$ Detector Temp: 320 $^{\circ}C$
Carrier: Helium Split ratio: 20/1
Relative peak area response of main component:
Initial analysis: 99.9%, $s = 0.01\%$ (7 sub samples in duplicate, November 2004)
Re-analysis: 99.9%, $s = 0.004\%$ (5 sub samples in duplicate, January 2008)

GC-FID: Instrument: Varian CP-3800
Column: VF-1MS, 30 m x 0.32 mm I.D. x 0.25 μ m
Program: 180 $^{\circ}C$ (1 min), 10 $^{\circ}C/min$ to 300 $^{\circ}C$ (3 min)
Injector: 250 $^{\circ}C$ Detector Temp: 320 $^{\circ}C$
Carrier: Helium Split ratio: 20/1
Relative peak area response of main component:
Initial analysis: Mean = 99.9%, $s = 0.01\%$ (5 sub samples in duplicate, January 2011)
Re-analysis: Mean = 100 %, $s = 0.004\%$ (5 sub samples in duplicate, November 2015)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction
(December 2004, 2005 and 2007)

Karl Fischer analysis: Moisture content < 0.1% mass fraction (December 2007, January 2011 &
November 2015)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.3 μ m
	Program:	80 °C, 15 °C/min to 100 °C, 15 °C/min to 300 °C (8 min)
	Injector:	200 °C
	Carrier:	Helium, 1.0 mL/min
		Transfer line temp: 320 °C Split ratio: 40/1
The retention time of the parent compound is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.		
Parent 11.6 min: 212 (M^+ , 100), 211 (7), 197 (22), 183 (5), 170 (7), 169 (52), 168 (8), 106 (5) m/z		
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1} , KBr powder
	Peaks:	3295, 2910, 2365, 2200, 2037, 1844, 1724, 1603, 1446, 1251, 1099, 1038, 922, 862, 805 and 768 cm^{-1}
^1H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Spectral data:	Solvent: CD_3OD (3.31 ppm) δ 2.80 (3H, s), 3.95 (3H, s), 6.90 (1H, dd, J = 2.2, 8.7 Hz), 7.08 (1H, d, J = 2.0 Hz), 7.82 (1H, d, J = 5.2 Hz), 8.02 (1H, d, J = 8.9 Hz), 8.14 (1H, d, J = 5.6 Hz) ppm
^{13}C NMR:	Instrument:	Bruker DMX-600
	Field strength:	150 MHz
	Spectral data:	Solvent: CD_3OD (49.0 ppm) δ 19.5, 56.0, 95.4, 110.9, 113.3, 116.4, 123.5, 130.1, 136.3, 138.0, 142.1, 144.2 and 162.6 ppm
Melting point:	264-266 °C	
Microanalysis:	Found: C = 73.5%; H = 5.8%; N = 13.2% (December, 2004)	
	Calc: C = 73.6%; H = 5.7%; N = 13.2% (Calculated for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$)	

Expiration of certification

The property values are valid till 16th November 2020, i.e. five years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body.

The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases, it is recommended that the end-user conduct their own in-house stability trials.

The long-term stability of the compound in solution has not been examined.

This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

Homogeneity assessment

The homogeneity of the material was assessed using purity assay by GC-FID on seven randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Metrological Traceability

The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis.

Recommended storage

When not in use, this material should be stored at or below 25 °C in a closed container in a dry, dark area.

Intended Use

For *in vitro* laboratory analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 4 December 2015

Characterisation data and property values specified in this report supersede those in all reports issued prior to 4th December 2015.