Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

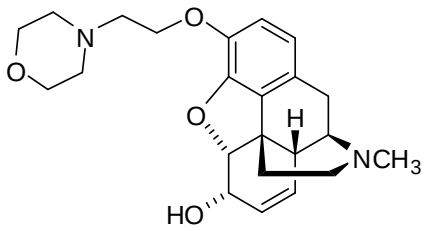
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D539b: Pholcodine

Report ID: D539b.2020.03

Chemical Formula: $C_{23}H_{30}N_2O_4$

Molecular Weight: 398.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
10-D-19	**509-67-1**	**95.5 ± 0.5%**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (5α,6α)-17-Methyl-3-[2-(4-morpholinyl)ethoxy]-7,8-didehydro-4,5-epoxymorphinan-6-ol.

Expiration of certification: The property values are valid till 22 December 2025, 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.

Description: Off-white powder sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use, this material should be stored at or below 25 ºC in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC-UV on ten 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.

Safety: Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
September 14, 2022.

This report supersedes any issued prior to 14 September 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see http://www.bipm.org).

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection at 212 nm, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

Supporting evidence is provided by headspace GC-MS analysis of occluded solvent, quantitative NMR, and elemental microanalysis. The purity estimate by qNMR was obtained using the two aromatic protons at 6.52 and 6.64 ppm, measured against a certified internal standard of dimethyl terephthalate

HPLC:	Instrument:	Waters 2695 Separation module or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Methanol: 20 mM NH_4OAc buffer, pH 6 (60:40)
	Flow rate:	1 mL/min
	Detector:	Waters PDA 2998 operating at 212 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 100.0%, s = 0.0% (5 sub samples in duplicate, March 2018)
	Re-analysis:	Mean = 100.0%, s = 0.0% (5 sub samples in duplicate, December 2020)
HPLC:	Instrument:	Shimadzu LC-20AB Binary pump, SIL-20A autosampler
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	30 °C
	Mobile Phase:	Water plus formic acid, pH 2.3: methanol (80:20)
	Flow rate:	0.3 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 282 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 100.0%, s = 0.02% (10 sub samples in duplicate, February 2011)
	Re-analysis:	Mean = 99.9%, s = 0.002% (5 sub samples in duplicate, March 2012)
	Re-analysis:	Mean = 99.7%, s = 0.09% (5 sub samples in duplicate, March 2015)
Thermogravimetric analysis:		Volatile content 4.2% and non-volatile residue < 0.2 % mass fraction (November 2010)
Karl Fischer analysis:		Moisture content ca.4.5% mass fraction (November 2010) Moisture content ca.4.5% mass fraction (February 2015) Moisture content ca.4.7% mass fraction (January 2018) Moisture content ca.4.4% mass fraction (December 2020)
QNMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Internal standard:	Dimethyl terephthalate (100% mass fraction)
	Initial analysis:	Mean = 95.9%, s = 0.31% (5 sub samples, December 2010)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Waters Acquity UPLC with TQD detector
	Operation:	Positive ion mode, direct infusion at 5-10 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV negative ion
	EM voltage:	670 V
	Cone voltage:	40 V
	Peak:	399.6 ($M+H^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	No occluded solvents were detected
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol/ethyl acetate/diethylamine (5/5/1) Single spot observed, R_f = 0.7. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3277, 2947, 2909, 2861, 2837, 1636, 1497, 1446, 1254, 1122, 1046, 968, 949, 760, 714, 652 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 1.85 (1H, m), 2.05 (1H, ddd, *J* = 5.1, 12.5, 12.5 Hz), 2.27 (1H, dd, *J* = 6.2, 18.7 Hz), 2.38 (1H, ddd, *J* = 3.6, 12.2, 12.2 Hz), 2.41 (3H, s), 2.50-2.59 (5H, m), 2.65 (1H, quintet, *J* = 2.9 Hz), 2.73 (1H, t, *J* = 5.9 Hz), 3.02 (1H, d, *J* = 18.7 Hz), 3.33 (1H, dd, *J* = 3.2, 6.1 Hz), 3.43 (1H, bd, *J* = 8.5 Hz) 3.72 (4H, t, *J* = 4.7 Hz), 4.10 (1H, ddd, *J* = 5.8, 5.8, 10.3 Hz), 4.15 (1H, bs), 4.23 (1H, ddd, *J* = 5.6, 5.6, 10.3 Hz), 4.86 (1H, dd, *J* = 1.2, 6.5 Hz), 5.26 (1H, ddd, *J* = 2.7, 2.7, 9.9 Hz), 5.67 (1H, m), 6.52 (1H, d, *J* = 8.2 Hz), 6.64 (1H, d, *J* = 8.2 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-400
	Field strength:	101 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 20.4, 35.7, 40.7, 42.9, 43.0, 46.3, 53.9, 57.7, 58.8, 66.5, 66.7, 66.9, 91.5, 115.4, 119.6, 127.9, 128.3, 131.4, 133.3, 140.8, 146.9 ppm
Melting point:		98-100 °C
Microanalysis:	Found:	C = 66.5%; H = 8.0%; N = 6.8% (December, 2010)
	Calculated:	C = 69.3%; H = 7.6%; N = 7.0% (Calculated for $C_{23}H_{30}N_2O_4$)
	Calculated:	C = 66.3%; H = 7.7%; N = 6.7% (Calculated for $C_{23}H_{30}N_2O_4.H_2O$)