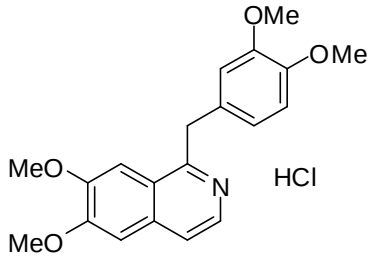
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D750b.2018.01 (Bottled 180629)
This batch of bottles was prepared from the bulk material on 29th June 2018.

Compound Name: **Papaverine hydrochloride**
Collection Number: D750b
Chemical Formula: $C_{20}H_{21}NO_4.HCl$
CAS Number: 61-25-6
Structure:

Description: White solid
Batch Number: 17-D-07
Molecular Weight: 375.8 (HCl), 339.4 (base)
Release Date: 28th September 2017



Synonyms: 1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline

Purity (mass fraction): 99.8 ± 1.9% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value by qNMR was obtained using a combination of the one-proton singlets at 7.3 and 7.4 ppm, the one-proton doublet at 7.9 ppm and the one-proton doublet at 8.1 ppm measured against a certified internal standard of dimethyl sulfone. Supporting evidence is provided by headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	200 °C (0.5 min), 6 °C/min to 280 °C (1 min), 20 °C /min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of main component:		
	Initial analysis:	Mean = 100%, s = 0.02% (10 sub samples in duplicate, July 2017)	
	Re-analysis:	Mean = 100%, s = 0.01% (5 sub samples in duplicate, July 2018)	
Thermogravimetric analysis:		Volatile content, 0.1% and non volatile residue < 0.2% mass fraction (June 2016)	
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (July 2017) Moisture content 0.13% mass fraction (June 2018)	
QNMR:	Instrument:	Bruker Avance-III-500	
	Field strength:	500 MHz	Solvent: D_2O (4.79 ppm)
	Internal standard:	Dimethyl sulfone (100% mass fraction)	
	Initial analysis:	Mean (7.3 & 7.4 ppm) = 99.6%, s = 0.3% (5 sub samples, November 2017)	
	Initial analysis:	Mean (7.9 ppm) = 99.5%, s = 0.4% (5 sub samples, November 2017)	
	Initial analysis:	Mean (8.1 ppm) = 99.8%, s = 0.2% (5 sub samples, November 2017)	

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro	
	Operation:	Positive ion mode, direct infusion at 10 μL/min	
	Ionisation:	ESI spray voltage at 3.2 kV positive ion	
	EM voltage:	650 V	
	Cone voltage:	45 V	
	Peak:	340.2 ($M+H^+$) *m/z*	
GC-MS:	Instrument:	Agilent 6890/5973	
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm	
	Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Transfer line temp: 280 °C
	Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1
	The retention time of the free base is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.		
	Free base (10.7 min): 339 (M^+, 74), 338 (100), 324 (89), 308 (22), 293 (11), 281 (11), 207 (19), 154 (13) *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:	Toluene, acetone	
TLC:	Conditions:	Kieselgel $60F_{254}$. Methanol / 33% aqueous ammonia (200/3) Single spot observed, R_f = 0.77. Visualisation with UV at 254 nm	
IR:	Instrument:	Biorad FTS300MX FT-IR	
	Range:	4000-400cm^{-1}, KBr powder	
	Peaks:	2500, 1994, 1955, 1635, 1608, 1508, 1436, 1409, 1282, 1146 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	500 MHz	Solvent: D_2O (4.79 ppm)
	Spectral data:	δ 3.63 (3H, s), 3.70 (3H, s), 3.84 (3H, s) 3.87 (3H, s), 6.72 (1H, dd, *J* = 1.8, 8.3 Hz), 6.79 (1H, d, *J* = 8.3 Hz), 6.91 (1H, d, *J* = 1.7 Hz), 7.21 (1H, s), 7.35 (1H, s), 7.82 (1H, d, *J* = 6.5 Hz), 8.10 (1H, d, *J* = 6.5 Hz) ppm	
	Acetone estimated at 0.03% mass fraction was observed in the ^{1}H NMR.		
^{13}C NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	126 MHz	Solvent: D_2O
	Spectral data:	δ 36.6, 55.6, 55.7, 56.3, 56.6, 104.6, 106.4, 112.1, 112.3, 121.7, 122.1, 122.2, 127.9, 129.5, 137.1, 147.8, 148.7, 151.9, 154.0, 156.4 ppm	
Melting point:		221.0-225.2 °C	
Microanalysis:		Found: C = 63.7%; H = 5.9%; N = 3.8%, Cl = 9.4% (August 2017) Calc: C = 63.9%; H = 5.9%; N = 3.7%, Cl = 9.4% (Calculated for $C_{20}H_{21}NO_4$.HCl)	

Expiration of certification
The property values are valid till 12th July 2021, i.e. three 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.

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by GC-FID 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.

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. 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).

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.

Intended use
This certified reference material may be used for instrument calibration.

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: 30 July, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 30th July 2018.