



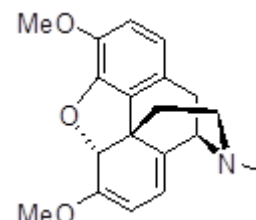
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D731b: Thebaine

Report ID: D731b.2022.01

Chemical Formula: C<sub>19</sub>H<sub>21</sub>NO<sub>3</sub>

Molecular Weight: 311.4 g/mol



### Certified value

Batch No.	CAS No.	Purity (mass fraction)
07-D-18	115-37-7	99.4 ± 0.7%

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

**IUPAC name:** (5 $\alpha$ )-3,6-Dimethoxy-17-methyl-6,7,8,14-tetrahydro-4,5-epoxymorphinan

**Expiration of certification:** The property values are valid till 12 September 2027, 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:** Light brown solid prepared by synthesis or sourced from an external supplier, 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 °C 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% confidence 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 with UV detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogenous 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.  
21 September 2022

This report supersedes any issued prior to 21 September 2022.

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

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

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## Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

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

Supporting evidence is provided by elemental microanalysis.

**HPLC:**

Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler  
 Column: X-Bridge C-18 5  $\mu\text{m}$  (4.6 mm x 150 mm)  
 Mobile Phase: Solvent A: 20 mM NH<sub>4</sub>OAc (pH 10) in Milli Q water  
 Solvent B: = Methanol  
 Gradient: 0-20 min 40% B, 20-22 min 40-55% B, 22-32 min 55% B, 32-35 min 55-40% B, 35-45 min 40% B  
 Flow Rate: 1.0 mL/min  
 Detector: Shimadzu SPD-M20A PDA, monitoring at 283 nm

Relative mass fraction of the main component:  
 Re-analysis: Mean = 99.7%, s = 0.007% (7 sub samples in duplicate, March 2009)  
 Re-analysis: Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, March 2010)  
 Re-analysis: Mean = 99.7%, s = 0.003% (5 sub samples in duplicate, February 2013)  
 Re-analysis: Mean = 99.7%, s = 0.009% (5 sub samples in duplicate, January 2018)  
 Re-analysis: Mean = 99.6%, s = 0.002% (5 sub samples in duplicate, September 2022)

**HPLC:**

Instrument: Waters Model 1525 Binary pump, 717 plus auto sampler  
 Column: Novapak C-18 5  $\mu\text{m}$  (3.9 mm x 150 mm)  
 Mobile Phase: Solvent A: 0.1% DEA in Milli Q water  
 Solvent B: 0.05% DEA in methanol  
 Gradient: 0-5 min 45% B, 5-15 min 45-70% B, 15-23 min 70% B, 23-24 min 70-45% B, 24-40 min 45% B  
 Flow Rate: 1.0 mL/min  
 Detector: Waters 996 PDA, monitoring at 283 nm

Relative mass fraction of the main component:  
 Initial analysis: Mean = 98.3%, s = 0.01% (10 sub samples in duplicate, June 2008)

**qNMR:**

Instrument: Bruker Avance-400  
 Field strength: 400 MHz  
 Solvent: DMSO-d<sub>6</sub>  
 Internal standard: Triphenyl phosphine oxide (100% m/m)  
 Purity estimate: 98.9% (mass fraction %, mean of five samples, s = 0.8 %, July 2008)

**Karl Fischer analysis:**

Moisture content ca. 0.4% mass fraction (March, 2010)  
 Moisture content ca. 0.23% mass fraction (February, 2013)  
 Moisture content ca. 0.22% mass fraction (January, 2018)  
 Moisture content ca. 0.22% mass fraction (September, 2022)

**Thermogravimetric analysis:** Initial volatile content < 0.1% and non-volatile residue < 0.2 % mass fraction

**Spectroscopic and other characterisation data**

ESI-MS:	Instrument:	Micromass Quattro Micro
	Operation:	Positive ion mode, direct infusion at 5 $\mu$ L/min
	Ionisation:	ESI capillary voltage at 3.2 KV positive ion
	EM voltage:	600 V
	Cone voltage:	29 V
	M/z:	312.2 ( $M^+H^+$ , 100%)
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Chloroform/methanol (90:10) Single spot observed, $R_f$ = 0.46. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 $cm^{-1}$ , KBr pellet
	Peaks:	2924, 2828, 2793, 1604, 1501, 1443, 1372, 1233, 1029, 907, 643 $cm^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker Tesla 600 MHz
	Field strength:	600 MHz
	Solvent:	CDCl <sub>3</sub> (7.26 ppm)
	Spectral data:	$\delta$ 1.72 (1H, m), 2.20 (1H, ddd, $J$ =12.6, 12.6, 5.2 Hz), 2.44 (3H, s), 2.61 (1H, dd, $J$ =12.9, 4.9 Hz), 2.65 (1H, dd, $J$ =18.0, 8.1 Hz), 2.81 (1H, ddd, $J$ =12.6, 12.6, 3.6 Hz), 3.30 (1H, d, $J$ =18.0 Hz), 3.59 (3H, s), 3.60 (1H, d, $J$ =8.3 Hz), 3.84 (3H, s), 5.04 (1H, d, $J$ =6.4 Hz), 5.29 (1H, s), 5.55 (1H, d, $J$ =6.4 Hz), 6.59 (1H, d, $J$ =8.0 Hz), 6.65 (1H, d, $J$ =8.0 Hz) ppm.
<sup>13</sup> C NMR:	Instrument:	Bruker Gyro-300
	Field strength:	75 MHz
	Solvent:	CDCl <sub>3</sub> (77.0 ppm)
	Spectral data:	$\delta$ 29.4, 37.0, 42.3, 46.0, 54.8, 56.4, 60.7, 77.1, 89.1, 95.8, 111.4, 112.9, 119.1, 127.7, 132.3, 133.3, 142.7, 144.7, 152.5 ppm
Melting point:		193-194 °C
Microanalysis:	Found:	C = 72.8 %; H = 6.9 %; N = 4.5% (November, 2007)
	Calculated:	C = 73.3 %; H = 6.8 %; N = 4.5% (Calculated for C <sub>19</sub> H <sub>21</sub> NO <sub>3</sub> )