



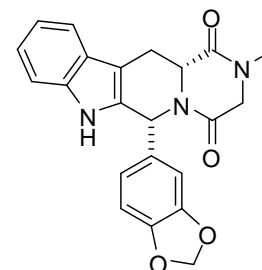
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D941: Tadalafil

Report ID: D941.2018.02

Chemical Formula: C₂₂H₁₉N₃O₄

Molecular Weight: 389.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
09-D-08	171596-29-5	99.1 ± 1.7%

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

IUPAC name: (6R, 12aR)-6-(benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazinol[1',2':1,-6]pyrido[3,4-b]indole-1,4-dione

Expiration of certification: The property values are valid till 21 June 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.

Description: Off-white powder 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 three 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 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.

Caution: Treat as a 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.
5 May 2020

This report supersedes any issued prior to 30 April 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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

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 ¹H 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_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

The purity estimate by qNMR was obtained using a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Column: X-Terra C-18, 5 µm (3.9 mm × 150 mm) Mobile Phase: Solvent A: Milli Q water Solvent B: acetonitrile Gradient 0-5 min 20% B, 5-28 min 20-80% B, 28-29 min 80% B, 29-30 min 80-20% B, 30-40 min 20% B Flow Rate: 1.2 mL/min Detector: UV at 283 nm Relative mass fraction of the main component: Initial analysis: Mean = 99.7%, s = 0.02% (10 sub samples in duplicate, June 2009) Re-analysis: Mean = 99.3%, s = 0.02% (5 sub samples in duplicate, June 2011) Re-analysis: Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, May 2012) Re-analysis: Mean = 99.1%, s = 0.05% (5 sub samples in duplicate, June 2015)
HPLC:	Column: Alltima C-18, 5 µm (4.6 mm × 150 mm) Mobile Phase: Solvent A: Milli Q water Solvent B: acetonitrile Gradient 0-15 min 35% B, 15-18 min 35-80% B, 18-23 min 80% B, 23-25 min 80-35% B, 25-35 min 35% B Flow Rate: 1 mL/min Detector: UV at 283 nm Relative mass fraction of the main component: Initial analysis: Mean = 99.3%, s = 0.03% (5 sub samples in duplicate, June 2018)
Karl Fischer analysis:	Moisture content 0.3% mass fraction (February 2009), Moisture content < 0.1% mass fraction (March 2010, July 2011, May 2012 and June 2015) Moisture content 0.14% mass fraction (June 2018)
Thermogravimetric analysis:	Initial volatile content < 0.1% and non volatile residue < 0.2 % mass fraction (April 2009)
QNMR:	Instrument: Bruker Avance 400 Field strength: 400 MHz Solvent: d ₆ -DMSO (2.50 ppm) Internal standard: Dimethyl terephthalate (100% mass fraction) Purity estimate: Mean = 99.7%, s = 0.9 (5 sub samples, May 2009)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Positive ion mode, direct infusion at 5 μ L/min
	Ionisation:	ESI spray voltage at 3.2 kV negative ion
	EM voltage:	650 V
	Cone voltage:	15 V
	Peak:	390 (M + H ⁺) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate/methanol (4/3/1) Single spot observed, R _f = 0.45. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm ⁻¹ , KBr powder
	Peaks:	3328, 2904, 1677, 1650, 1490, 1437, 1324, 1269, 1242, 1152, 1042, 940, 746 cm ⁻¹
¹ H NMR:	Instrument:	Avance 400
	Field strength:	400 MHz
	Solvent:	CDCl ₃ (7.26 ppm)
	Spectral data:	δ 3.04 (3H, s), 3.21 (1H, ddd, <i>J</i> = 1.4, 11.6, 16.0 Hz), 3.78 (1H, dd, <i>J</i> = 4.4, 16 Hz), 3.93 (1H, d, <i>J</i> = 17.4 Hz), 4.11 (1H, dd, <i>J</i> = 1.5, 17.5 Hz), 4.30 (1H, dd, <i>J</i> = 4.2, 11.4 Hz), 5.85 (1H, d, <i>J</i> = 8.9 Hz), 5.86 (1H, d, <i>J</i> = 8.9 Hz), 6.14 (1H, s), 6.68 (1H, d, <i>J</i> = 8.1 Hz), 6.73 (1H, d, <i>J</i> = 1.7 Hz), 6.84 (1H, dd, <i>J</i> = 1.7, 8.1 Hz), 7.17 (2H, m), 7.27 (1H, m), 7.61 (1H, m), 7.95 (1H, s) ppm
¹³ C NMR:	Instrument:	Bruker Gyro-300
	Field strength:	75 MHz
	Solvent:	CDCl ₃ (77.0 ppm)
	Spectral data:	δ 23.8, 33.6, 52.1, 56.2, 56.7, 101.1, 106.5, 107.4, 108.2, 111.2, 118.6, 120.1, 120.7, 122.5, 126.2, 132.7, 135.3, 136.5, 147.1, 147.8, 166.4, 166.8 ppm
Microanalysis:	Found:	C = 67.7%; H = 4.7%; N = 10.8% (February 2009)
	Calculated:	C = 67.9%; H = 4.9%; N = 10.8% (Calculated for C ₂₂ H ₁₉ N ₃ O ₄)
Specific rotation	[α] _D = + 69.4° (July 2009)	