Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

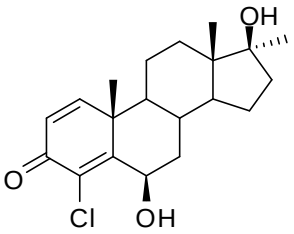
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D615b: 6β-Hydroxyturinabol

Report ID: D615b.2021.01 (Ampouled 170928)

Chemical Formula: $C_{20}H_{27}ClO_3$

Molecular Weight: 350.9 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
17-S-05	25486-01-5	967 ± 16 µg

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

IUPAC name: (6β,17β)-4-Chloro-6,17-dihydroxy-17-methylandrosta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 31 August 2024, 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D615b. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

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

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 967 ± 16 µg of anhydrous 6β-hydroxyturinabol. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

Recommended storage: When not in use, this material should be stored at or below 4 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%.

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.

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Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on seven randomly selected ampoules 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.
10 September 2021

This report supersedes any issued prior to 10 September 2021.

Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters 1525 Binary pump, 717 auto sampler or Waters Alliance 2695
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-22 min 30% B; 22-24 min 30-80% B; 24-28 min 80% B; 28-30 min 80-30% B, 30-35 min 30% B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 252 nm Shimadzu SPD-M20A PDA operating at 252nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 96.9%, s = 0.12% (7 ampoules in duplicate, October 2017)
	Re-analysis:	Mean = 96.8%, s = 0.04% (5 ampoules in duplicate, October 2018)
	Re-analysis:	Mean = 96.7%, s = 0.05% (5 ampoules in duplicate, August 2021)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, 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 qualitative headspace GC-MS analysis of occluded solvents andelemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-22 min 30% B; 22-24 min 30-80% B; 24-28 min 80% B; 28-30 min 80-30% B, 30-35 min 30% B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 252 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 96.6%, s = 0.05% (7 sub samples in duplicate, September 2017)
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (September 2017)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (September 2017). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	220 °C (1 min), 8 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times of the parent compound and *bis*-TMS derivative are 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 (11.1 min):	332 (16), 314 (16), 299 (30), 297 (28), 279 (26), 248 (30), 239 (25), 223 (24), 193 (30), 191 (37), 171 (100), 147 (65), 119 (55), 107 (87), 91 (82), 79 (78), 77 (77) *m/z*
	Bis-TMS (11.4 min):	479 (1), 317 (15), 315 (38), 281 (5), 279 (8), 243 (21), 143 (100), 75 (86), 73 (60) *m/z*
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:	35 V
	Peak:	373.1 (M^{Cl35}+Na^+), 375.1 (M^{Cl35}+Na^+) *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
	Split ratio:	50/1
	Solvents detected:	Ethyl acetate, chloroform and dichloromethane
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (1:1) Single spot observed, R_f = 0.35. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3427, 3352, 3213, 2960, 2929, 2871, 2837, 1663, 1369, 1159, 1084, 1046, 968, 930, 836, 565 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (3.31 ppm)
	Spectral data:	δ 0.97 (3H, s), 1.11 (1H, m), 1.17-1.46 (5H, m), 1.20 (3H, s), 1.52 (3H, s) 1.56-1.70 (3H, m), 1.70-1.89 (4H, m), 2.07-2.18 (2H, m), 5.51 (1H, t, *J* = 3.1 Hz), 6.33 (1H, d, *J* = 10.2 Hz), 7.10 (1H, d, *J* = 10.2 Hz) ppm
		Ethyl acetate estimated at 0.6% mass fraction was observed in the ^{1}H NMR.^{13}C NMR:
	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.19 ppm)
	Spectral data:	δ 14.2, 21.0, 22.7, 23.5, 26.0, 30.7, 31.5, 38.9, 39.0, 45.7, 46.5, 50.0, 51.8, 68.7, 81.6, 125.4, 130.5, 156.8, 159.0, 178.7 ppm
Melting point:		269-274 °C
Microanalysis:	Found:	C = 68.2%; H = 8.0%; Cl = 9.9% (September 2017)
	Calculated:	C = 68.5%; H = 7.8%; Cl = 10.1% (Calculated for $C_{20}H_{27}ClO_3$)