Australian Government

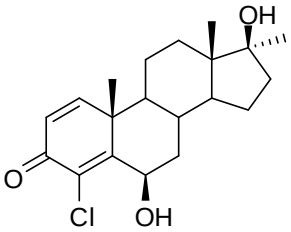
National Measurement Institute

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D615b.2018.01 (Ampouled 170928)
This batch of ampoules was prepared from the bulk material on 28th September 2017.

Compound Name: **6β-Hydroxyturinabol**
Collection Number: D615b
Chemical Formula: $C_{20}H_{27}ClO_3$
CAS Registry Number: 25486-01-5
Structure:

Description: Off white solid
Batch Number: 17-S-05
Molecular Weight: 350.9
Release date: 29th October 2017



Synonyms: 4-Chloro-6β,17β-dihydroxy-17-methyl-androst-1,4-dien-3-one
6β-Hydroxy-4-chlorodehydromethyltestosterone

The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D615b. 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 uncertainty is stated at the 95% coverage interval.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters 1525 Binary pump, 717 auto sampler
	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 or Waters 2998 PDA operating at 252 nm
	Relative mass fraction of 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 sub samples in duplicate, October 2018)

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

The purity value was obtained from a combination of traditional analytical techniques by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. Supporting evidence is provided by headspace GC-MS analysis of occluded solvents and elemental 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 main component:	
	Initial analysis:	Mean = 96.6%, s = 0.05% (7 sub samples in duplicate, 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
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (September 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:	35 V
	Peak:	373.1 and 375.1 ($M+Na^+$) *m/z*
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 Transfer line temp: 280 °C
	Carrier:	Helium, 1.0 mL/min Split ratio: 20/1
	The retention times of the parent compound and *bis*-TMS derivative are reported along 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*
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:	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: $CDCl_3$ (3.31ppm)
	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) Calc: C = 68.5%; H = 7.8%; Cl = 10.1% (Calculated for $C_{20}H_{27}ClO_3$)

Expiration of certification
The property values are valid till 25th October 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 ampoules 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 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.

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.

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.

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: 27 November, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 27th November 2018.