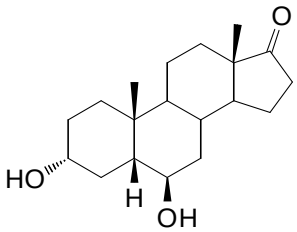
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D867.2016.01 (Ampouled 110120)
This batch of ampoules was prepared from the bulk material on 20th January 2011.

Compound Name: **6β-Hydroxyetiocholanolone**
Collection Number: D867
Chemical Formula: $C_{19}H_{30}O_3$
CAS Registry Number: 14357-02-9
Structure:



Description: White powder
Batch Number: 03-S-08
Molecular Weight: 306.4
Batch production completed: November 2004

Synonyms: 3α, 6β-Dihydroxy-5β-androstan-17-one

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 D867. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 991 ± 11 ug of anhydrous 6β-hydroxyetiocholanolone. The uncertainty is stated at the 95% coverage interval.

GC-FID:	Instrument:	Varian CP-3800	
	Column:	VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 10 °C/min to 240 °C, 30 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.9%, s = 0.008% (7 ampoules in duplicate, March 2011)	
	Re-analysis:	Mean = 98.8%, s = 0.03% (5 ampoules in duplicate, January 2014)	
	Re-analysis:	Mean = 98.7%, s = 0.02% (5 ampoules in duplicate, November 2016)	

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

Purity estimate obtained from a combination of traditional analytical techniques. 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. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 240 °C (10 min), 30 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.9%, s = 0.10% (5 sub samples in duplicate, November 2010)	
GC-FID:	Instrument:	Varian CP-3800	
	Column:	VF-1, 30 m x 0.32 mm I.D. x 0.25 µm	
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min) 40 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.9%, s = 0.10% (7 sub samples in duplicate, May 2004)	
	Re-analysis:	Mean = 98.4%, s = 0.11% (5 sub samples in duplicate, October 2007)	
Thermogravimetric analysis:		Volatile content < 0.1% and non-volatile content < 0.2% mass fraction (October 2007)	
Karl Fischer analysis:		Moisture content < 0.4% mass fraction (October 2007 & November 2010)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973	
Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 µm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

Tris-TMS derivative:

Instrument:	Agilent 6890/5973	
Column:	HP Ultra 1, 17 m × 0.2 mm I.D. × 0.11 µm	
Program:	189 °C (0.2 min), 3 °C /min to 240 °C, 10 °C /min to 265 °C, 30 °C/min to 310 °C (2 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 14/1

The retention times of the parent compound and the *tris*-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 (10.0 min): 306 (M^+, 4), 288 (8), 273 (47), 233 (100), 199 (6), 159 (6), 95 (20), 79 (13), 67 (13), 55 (13), 41 (9) m/z

Tris-TMS (11.7 min):522 (59), 507 (19), 417 (21), 377 (38), 327 (52), 169 (21), 147 (21), 73 (100) m/z

TLC: Conditions: Kieselgel 60F_{254}. 100% Ethyl acetate, Single spot observed, R_f = 0.21, Visualization with vanillin, H_2SO_4 spray

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3478, 3398, 2930, 1734, 1456, 1413, 1369, 1323, 1300, 1253, 1225, 1171, 1042, 1004, 921, 611 cm^{-1}

^{1}H NMR:

Instrument:	Gyro 300	
Field strength:	300 MHz	Solvent: CD_3OD (3.3 ppm)
Key spectral data:	δ 0.88 (3H, s), 1.05 (1H, ddd, *J* = 3.0, 14.3, 14.3 Hz), 1.14 (3H, s), 2.45 (1H, dd, *J* = 8.7, 19.2 Hz), 3.62 (1H, m), 3.82 (1H, m), ppm	

^{13}C NMR:

Instrument:	Bruker Advance 300	
Field strength:	75.5 MHz	Solvent: CD_3OD (49 ppm)
Spectral data:	δ 13.8, 19.9, 21.8, 25.5, 30.1, 30.5, 31.6, 33.5, 34.5, 35.8, 35.9, 36.3, 41.0 47.9, 48.8, 51.4, 71.1, 72.8, 221.1 ppm	

Melting point: 221-223 °C

Microanalysis: Found: C = 74.5%, H = 9.6% (April 2005)
Calc: C = 74.5%, H = 9.9% (Calculated for $C_{19}H_{30}O_3$)

Expiration of certification
The property values are valid till 3rd November 2021, 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 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.

This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by GC-FID 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
For ***in vitro*** laboratory analysis only.

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: 1 December, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 1st December 2016.

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