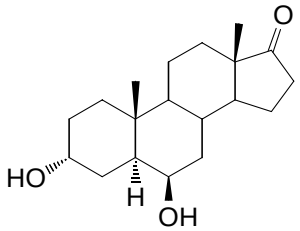
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D886.2011.02

Compound Name: **3α, 6β-Dihydroxy-5α-androstan-17-one**
Description: White powder
Collection No.: D886 Batch No: 03-S-07
Chemical Formula: $C_{19}H_{30}O_3$ Molecular Weight: 306.4
CAS Registry No: 152886-16-3 Batch production completed: April 2005
Structure:



Purity (mass fraction): 89.9 ± 0.8% (95% coverage interval)

Purity estimate obtained by subtraction from 100% of GC-FID, thermogravimetric analysis and Karl Fischer.

GC-FID
Instrument: Agilent 6890N
Column: HP-1, 29.9 m x 0.32 mm I.D. x 0.25 µm
Program 180 °C (1 min), 40 °C/min to 230ºC (12 min), 25ºC/min to 300 °C (2 min)
Injector Temp: 250 °C Detector Temp: 320 °C
Carrier: Helium Split ratio: 20/1
Relative peak area response of main component:
Initial analysis: Mean = 94.6%, s = 0.10% (7 sub samples in duplicate, May 2005)
Re-analysis: Mean = 94.8%, s = 0.04% (5 sub samples in duplicate, August 2011)

GC-MS:
Parent compound:
Instrument: HP6890/5973
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: Ultra 1, 17 m x 0.2 mm I.D.x 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 observed in the mass spectrum. The latter are reported in mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.53 min): 306 (M^+, 87), 288 (16), 270 (36), 255 (31), 230 (26), 213 (34), 199 (26), 150 (36), 147 (28), 145 (29), 123 (46), 109 (48), 107 (58), 105 (47), 95 (100), 91 (59), 81 (53), 79 (74), 67 (72), 55 (77) m/z

Tris-TMS (11.9 min): 522 (34), 507 (16), 417 (26), 327 (45), 237 (5), 221 (5), 195 (8), 182 (13), 169 (21), 147 (8), 129 (10), 105 (8), 91 (7), 73 (100) m/z.

TLC:	Conditions:	Kieselgel $60F_{254}$. 100% Ethyl acetate, single spot observed, $R_f = 0.28$, visualization with vanillin, H_2SO_4 spray
IR:	Instrument:	BioRad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3571, 3434, 2945, 2922, 2856, 1715, 1613, 1434, 1368, 1244, 1024 cm^{-1}
^1H NMR:	Instrument:	Bruker DMX 300
	Field strength:	300 MHz Solvent: CD_3OD
	Key spectral data:	δ 0.78-0.90 (1H, m), 0.90 (3H, s), 1.03 (3H, s), 1.15-1.45 (7H, m), 1.50-1.82 (6H, m), 1.85-2.15 (5H, m), 2.43 (1H, dd, *J* = 8.3, 18.0 Hz), 3.70 (1H, m), 4.06 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX 300
	Field strength:	75.5 MHz Solvent: CD_3OD
	Spectral data:	δ 14.2, 15.3, 21.0, 22.7, 29.6, 31.3, 32.8, 34.1, 35.2, 36.7, 37.4, 39.6, 43.3, 49.2, 52.6, 55.9, 67.3, 72.4, 224.1 ppm
Microanalysis:		Found: C = 70.5%, H = 9.5%; Calc: C = 74.5%, H = 9.9%
Melting point:		N/A
Thermogravimetric analysis:		Volatile content 3.64%. Non-volatile content < 0.2% mass fraction (August 2008).
Karl Fischer analysis:		Moisture content c.a. 5.7% mass fraction (August 2008 &2011).

Expiration of certification
The property values are valid till 22nd August 2016, 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/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 5 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 7 randomly selected 1 mg samples of the material. The material was judged to be 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.

Recommended storage
When not in use this material should be stored at or below 20 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: 18 July, 2012

Characterisation data and property values specified in this report supersede those in all reports issued prior to 18th July 2012.

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