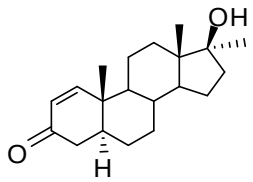
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D904.2017.01 (Ampouled 110627)
This batch of ampoules was prepared from the bulk material on 27th June 2011.

Compound Name: **17β-Hydroxy-17α-methyl -5α-androst-1-ene-3-one**
Description: White crystals
Collection Number: D904
Chemical Formula: $C_{20}H_{30}O_2$
CAS Registry Number: 65-04-3
Batch Number: 06-S-01
Molecular Weight: 302.5
Release date: 10th June 2013
Structure:



Synonyms: Methyl-1-testosterone
17α-Methyl-1-testosterone
17α-Methyl-17β-hydroxy-Δ1−5α-androsten-3-one
17β-Hydroxy-17-methyl-5α-androst-1-en-3-one
17β-Hydroxy-17α-methyl-5α-androst-1-en-3-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 D904. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 994 ± 18 µg of anhydrous 17β-hydroxy-17α-methyl -5α-androst-1-ene-3-one. The uncertainty is stated at the 95% coverage interval.

This reference material has NOT been extensively quantified by the Chemical Reference Materials team, NMI. This material should be considered for use in qualitative analysis only.

GC-FID:	Instrument:	Agilent 6890/Varian CP-3800	
	Column:	HP-1 or VF-1MS, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 20 °C/min to 250 °C (5 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 = 99.6%, s = 0.01% (7 ampoules in duplicate, June 2011)	
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, April 2014)	
	Re-analysis:	Mean = 99.6%, s = 0.04% (5 ampoules in duplicate, March 2017)	

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 and Karl Fischer analysis. Supporting evidence is provided by elemental microanalysis, thermogravimetric analysis and ^{1}H NMR.

GC-FID:	Instrument:	HP5890	
	Column:	ZB-1, 28.5 m × 0.32 mm I.D. × 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 = 99.7%, s = 0.01% (10 sub samples in duplicate, February 2006)	
GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 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 = 99.8%, s = 0.08% (5 sub samples in duplicate, February 2007)	
GC-FID:	Instrument:	Varian 3800	
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, January 2008)	
GC-FID:	Instrument:	Varian 3800	
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 20 °C/min to 250 °C (5 min), 40 °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 = 99.7%, s = 0.01% (5 sub samples in duplicate, February 2009)	
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (February 2006 & February 2007 & February 2009)	
Karl Fischer analysis:		Moisture content 0.2% mass fraction (January 2008) Moisture content < 0.8% mass fraction (February 2009)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP5890/5971A	
Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm	
Program:	220 °C (2 min), 10 °C/min to 300 °C (7 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

Bis-TMS derivative:

Instrument:	Agilent 6890/5973	
Column:	HP Ultra 1, 17 m × 0.20 mm I.D. × 0.10 μm	
Program:	200 °C, 10 °C/min to 250 °C, 20 °C/min to 310 °C (2 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 12/1

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 (8.23 min): 302 (M^+, 16), 245 (10), 160 (19), 149 (15), 134 (25), 122 (100), 107 (58), 91 (40), 81 (30), 71 (48) m/z

Bis-TMS (5.1 min): 446 (M^+, 21), 431 (11), 356 (7), 246 (6), 194 (32), 179 (17), 163 (8), 143 (100), 73 (74) m/z

TLC: Conditions: Kieselgel 60F_{254}. Ethyl acetate/Chloroform (1/4)
Single spot observed, R_f = 0.28. Visualisation with UV at 254 nm

IR: Instrument: Biorad FTS300MX FT-IR
Range: 4000-400cm^{-1}, KBr powder
Peaks: 3517, 3336, 2928, 2868, 1664, 1597, 1471, 1450, 1439, 1368, 1272, 1163, 1084, 1066, 936, 782 cm^{-1}

^{1}H NMR: Instrument: Bruker DMX-500
Field strength: 500 MHz Solvent: $CDCl_3$ (7.26 ppm)
Spectral data: δ 0.88 (3H, s), 0.90-0.99 (2H , m), 1.02 (3H, s), 1.18-1.59 (10H , m), 1.21 (3H , s), 1.70-1.76 (2H, m), 1.78-1.83 (2H, m), 1.90 (1H, m), 2.21 (1H, dd, *J* = 4.0, 17.8 Hz), 2.36 (1H, dd, *J* = 14.2, 17.8 Hz), 5.84 (1H, dd, *J* = 0.7, 10.2 Hz), 7.14 (1H, d, *J* = 10.2 Hz) ppm

^{13}C NMR: Instrument: Bruker Gyro-300
Field strength: 75 MHz Solvent: $CDCl_3$ (77.2 ppm)
Spectral data: δ 13.0, 14.1, 20.9, 23.1, 25.8, 27.5, 31.0, 31.5, 36.5, 38.9, 39.0, 40.9, 44.4, 45.6, 50.0, 50.6, 81.5, 127.4, 158.3, 200.1 ppm

Melting point: 152-153 °C

Microanalysis: Found: C = 79.6 %; H = 9.8 % (March, 2006)
Calc: C = 79.4 %; H = 10.0 % (Calculated for $C_{20}H_{30}O_2$)

Expiration of certification
The property values are valid till 22nd March 2020, 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.

This material has demonstrated stability over a minimum period of three 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.

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: 29 March, 2017.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 29th March 2017.