Australian Government

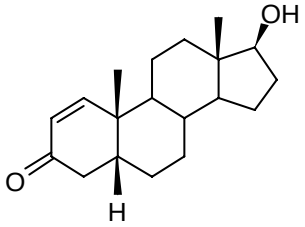
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D564.2011.02 (Ampouled 081203)
This batch of ampoules was prepared from the bulk material on 3rd December 2008.

Compound Name: **5β-Androst-1-en-17β-ol-3-one**
Collection Number: D564
Chemical Formula: $C_{19}H_{28}O_2$
CAS Registry Number: 10529-96-1
Structure:

Description: White crystals
Batch Number: 99-000015
Molecular Weight: 288.4
Batch production completed: February 1999
Metabolite of boldenone.



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 D564. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 1.009 ± 0.012 mg of anhydrous 5β-androst-1-en-17β-ol-3-one. The uncertainty is stated at the 95% coverage interval.

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 29.93 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 15 °C/min to 250 °C (5 min), 30 °C/min to 300 °C (3min)	
	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 = 0.007% (5 ampoules in duplicate, February 2011)	
GC-FID:	Instrument:	Varian 3800	
	Column:	VF-1MS Capillary, 29.82 m x 0.32 mm I.D. x 0.25 µm	
	Program:	180 °C (1 min), 15 °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 = 99.9% (7 sub samples, February 1999)	
	Re-analysis:	Mean = 99.6%, s = 0.008% (7 ampoules in duplicate, December 2008)	
	Re-analysis	Mean = 99.5%, s = 0.004% (5 ampoules in duplicate, February 2010)	

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 and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 5890	
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 30 °C/min to 300 °C (3min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.9% (7 subsamples, February 1999)	
	Re-analysis:	Mean = 99.5%, s = 0.02 (8 subsamples in duplicate, June 2006)	
HPLC:	Peak area percentage of total > 99.5% (3 sub samples)		
	Column:	Alltima C18, 5 µm (4.6 mm × 150 mm)	
	Mobile Phase:	Acetonitrile/ water (60:40)	
	Flow Rate:	1 mL/min	
	Detector:	Refractive index	
	Retention time:	6.03 min	
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (March 1999 & October 2005).	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP6890 / 5973	
Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 0.10 µm	
Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)	
Injector:	280 °C, Split injection	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Scan range: 50-550 m/z

Bis-TMS derivative:

Instrument:	HP 6890/5973	
Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 µm	
Program:	170 °C (0.5 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)	
Injector:	280 °C, Split injection	Transfer line temp: 300 °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 (5.9 min): 288 (M^+, 20), 270 (10), 134 (31), 122 (100), 109 (78), 78 (29) m/z
Bis-TMS (7.6 min): 432 (M^+, 36), 417 (34), 206 (22), 194 (100), 73 (73) m/z

TLC: Conditions: Kieselgel 60F_{254}. Hexane /ethyl acetate (5:3)
Single spot observed, R_f = 0.25 (5 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3426, 1673, 1450, 1273, 1056, 842, 784 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Advance-300	
Field strength:	300 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.77 (3H, s), 1.21 (3H, s), 3.64 (1H, t), 5.89 (1H, d), 6.83 (1H, d) ppm	

^{1}Instrument: Bruker Advance-300

Field strength:	75 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 11.5, 21.2, 22.3, 23.7, 25.9, 26.7, 30.6, 35.6, 37.0, 39.0, 39.3, 41.3, 43.4, 46.8, 50.7, 82.0, 127.3, 162.0, 201.2 ppm	

Melting point: 201.5 – 202.5 °C

Microanalysis: Found: C = 79.0%; H = 9.8% (February 1999)
Calc: C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)

Expiration of certification
The property values are valid till 1st February 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 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 3 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 five randomly selected ampoules 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 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: 16 July, 2012.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 16th July 2012.