Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

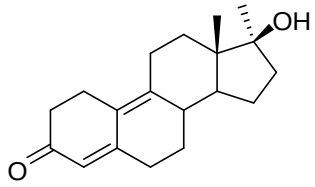
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D916b: Methyldienolone

Report ID: D916b.2019.01 (Ampouled 170727)

Chemical Formula: $C_{19}H_{26}O_2$

Molecular Weight: 286.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
16-S-011	14531-89-6	917µg

IUPAC name: 17β-Hydroxy-17α-methyl-estra-4,9-dien-3-one

Expiration of certification: The property values are valid till 23 January 2022, 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 material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: 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 D916b. Material was prepared by synthesis , and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. toluene). This will transfer approximately 917 µg of anhydrous methyldienolone.

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.

Stability: In the absence of long term 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. The long-term stability of the compound in solution has not been examined.

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.

Safety: Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
18 February 2019

This report supersedes any issued prior to 18 February 2019

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)	
	Injector:	200 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component:		
	Initial analysis:	Mean = 98.4%, s = 0.3% (7 ampoules in duplicate, August 2017)	
	Re-analysis:	Mean = 92.2%, s = 0.3% (5 ampoules in duplicate, August 2018)	
	Re-analysis:	Mean = 92.5%, s = 0.1% (5 ampoules in duplicate, January 2019)	

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

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by HPLC with UV detection, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (1 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component:		
	Initial analysis:	Mean = 99.0%, s = 0.03% (7 sub samples in duplicate, November 2016)	
HPLC:	Instrument:	Thermo Scientific Ultimate 3000 RS pump, RS autosampler	
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)	
	Column oven:	40 °C	
	Mobile Phase:	Acetonitrile/MilliQ water (45:55 v/v)	
	Flow rate:	1.0 mL/min	
	Detector:	RS Diode Array Detector operating at 310 nm	
	Relative peak area of main component:		
	Initial analysis:	Mean = 99.5%, s = 0.01% (7 sub samples in duplicate, December 2016)	
Thermogravimetric analysis:		Volatile content 0.2% and non volatile residue < 0.2% mass fraction	
Karl Fischer analysis:		Moisture content 0.2% mass fraction (November 2016)	

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Positive ion mode, direct infusion at 2 µL/min
	Ionisation:	ESI spray voltage at 3.2 kV positive ion
	EM voltage:	500 V
	Cone voltage:	30 V
	Peak:	287 ($M+H^+$) *m/z*
GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 2, 17 m × 0.20 mm I.D. × 0.25 µm
	Program:	80 °C (0.5 min), 50 °C to 200 °C (0.5 min), 15 °C to 310 °C (5 min)
	Injector:	290 °C
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	30/1
	Bis-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm
	Program:	180 °C (0.5 min), 12 °C/min to 310 °C (3 min)
	Injector:	260 °C
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	30/1
	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 to charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (7.7 min):	286 (M+, 100), 268 (21), 253 (35), 228 (91), 215 (88), 199 (22), 186 (14), 174 (24), 160 (65), 129 (29), 117 (38), 91 (45), 77 (20), 55 (14), 43 (48) *m/z*
	Bis-TMS (13.5 min):	430 (M+, 100), 415 (7), 325 (13), 285 (24), 246 (6), 143 (3), 73 (23) *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:	Benzene
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr pellet
	Peaks:	3498, 2941, 2862, 1644, 1608, 1450, 1388, 1278, 1215, 1166, 1069, 955, 856, 728 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Spectral data:	δ 1.01 (3H, s), 1.21 (3H, s), 1.23-1.28 (1H, m), 1.32-1.44 (3H, m), 1.58-1.66 (2H, m), 1.74-1.81 (1H, m), 1.85-1.93 (2H, m), 2.12 (1H, bm), 2.25 (1H, bm), 2.32-2.47 (4H, m), 2.53 (1H, m), 2.82 (1H, dd, *J* = 16.1, 2.5 Hz), 2.89 (1H, ddd, *J* = 14.6, 4.9, 4.9 Hz), 5.66 (1H, s) ppm Benzene estimated at 1% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	150 MHz Solvent: $CDCl_3$ (77 ppm)
	Spectral data:	δ 13.3, 23.3, 25.7, 25.8, 25.8, 27.3, 30.9, 31.5, 37.0, 38.9, 40.2, 45.3, 50.9, 81.3, 122.1, 125.5, 146.3, 157.3, 199.8 ppm
Melting point:		159-161 °C
Microanalysis:	Found:	C = 79.6%; H = 9.2% (November 2016)
	Calculated	C = 79.7%; H = 9.2% (Calculated for $C_{19}H_{26}O_2$ containing 1% benzene and 0.2% water)