Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

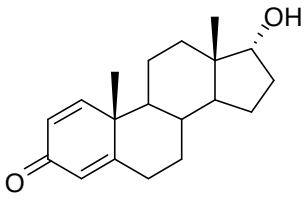
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D582b: 17α-Boldenone

Report ID: D582b.2021.03 (Ampouled 150310)

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

Molecular Weight: 286.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
04-S-06	**27833-18-7**	**986 ± 25 μg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (17α)-17-Hydroxyandrosta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 31 March 2026, 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D582b. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material may be used for instrument calibration.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 986 ± 25 μg of anhydrous 17α-boldenone. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

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.

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.
27 July 2022

This report supersedes any issued prior to 27 July 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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 or 7890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 30 °C/min to 250 °C (7 min), 30 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.4%, s = 0.03% (7 ampoules in duplicate, March 2015)	
	Re-analysis:	Mean = 99.2%, s = 0.03% (5 ampoules in duplicate, March 2016)	
	Re-analysis:	Mean = 99.3%, s = 0.1% (5 ampoules in duplicate, October 2017)	
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, September 2018)	
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, April 2021)	

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

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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

Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 30 °C/min to 250 °C (7 min), 30 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.4%, s = 0.07% (7 sub samples in duplicate, March 2015)	
Karl Fischer analysis:		Moisture content 0.1% mass fraction (March 2015)	
Thermogravimetric analysis:		Volatile content 0.1% and non volatile residue < 0.2 % mass fraction (October 2009)	

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
	Bis-TMS derivative:		
	Instrument:	Agilent 6890/5973	
	Column:	HP-5MS, 30 m x 0.25 mm I.D.x 0.25 µm	
	Program:	185 °C (0.2 min), 3 °C/min to 236 °C, 10°C/min to 265 °C, 30 °C/min to 310 °C (1 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 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 (8.3 min):	286 (M^+, 3), 268 (5), 207 (4), 147 (23), 122 (100), 91 (27) *m/z*	
	Bis-TMS (10.2 min):	430 (M^+, 28), 325 (34), 206 (77), 191 (22), 73 (100) *m/z*	
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (3/1) Single spot observed, R_f = 0.16. Visualisation with UV at 254 nm	
IR:	Instrument:	Biorad FTS3000MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	3491, 2942, 1655, 1617, 1599, 1458, 1377, 1284, 1245, 1051, 897, 829 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker DMX-500	
	Field strength:	500 MHz	Solvent: $CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.72 (3H, s), 1.03-1.13 (2H, m), 1.16-1.26 (1H, m), 1.22 (3H, s), 1.34-1.83 (9H, m), 1.97 (1H, m), 2.15 (1H, m), 2.34 (1H, m), 2.46 (1H, m), 3.74 (1H, d, *J* = 5.9 Hz), 6.06 (1H, s), 6.21 (1H, dd, *J* = 1.8, 10.1 Hz), 7.06 (1H, d, *J* = 10.1 Hz) ppm	
^{13}C NMR:	Instrument:	Bruker DMX-500	
	Field strength:	151 MHz	Solvent: $CDCl_3$ (77.2 ppm)
	Spectral data:	δ 16.9, 18.7, 22.3, 24.7, 31.0, 32.3, 32.8, 33.9, 35.7, 43.6, 45.3, 47.7, 52.1, 79.4, 123.7, 127.4, 156.0, 169.4, 186.4 ppm	
Melting point:		188-189 °C	
Microanalysis:	Found:	C = 79.5%; H = 9.1% (November 2004)	
	Calculated:	C = 79.7%; H = 9.2% (Calculated for $C_{19}H_{26}O_2$)	