Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

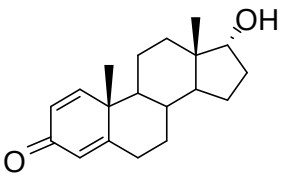
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D582b: 17α-Boldenone

Report ID: D582b.2020.01

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

Molecular Weight: 286.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
04-S-06	27833-18-7	99.4 ± 0.7%

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

IUPAC name: 17α-Hydroxyandrost-1,4-dien-3-one.

Expiration of certification: The property values are valid till 1 April 2030, i.e. ten 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 that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White solid prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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: 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 ten randomly selected 1-2 mg sub samples 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 a 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.
23 April 2020

This report supersedes any issued prior to 23 April 2020

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:

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 qualitativeelemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 7 °C/min to 220 °C, 20 °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 = 99.7%, s = 0.02% (10 sub samples in duplicate, November 2004)
	Re-analysis:	Mean = 99.6%, s = 0.03% (5 sub samples in duplicate, September 2009)
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)
	Re-analysis:	Mean = 99.5%, s = 0.07% (5 sub samples in duplicate, April 2020)
Karl Fischer analysis:		Moisture content 0.2% mass fraction (September 2009) Moisture content 0.1% mass fraction (March 2015 & April 2020)
Thermogravimetric analysis:		Volatiles 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
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	HP 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
	Split ratio:	12/1
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	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 (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.
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr pellet
	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 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 $C_{19}H_{26}O_2$)