Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

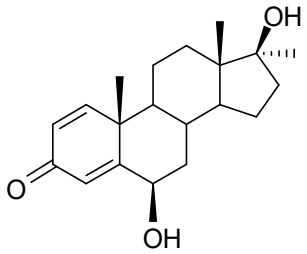
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D565: 6β-Hydroxymethandienone

Report ID: D565.2026.01 (Ampouled 180913)

Chemical Formula: $C_{20}H_{28}O_3$

Molecular Weight: 316.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-000016	33526-41-9	1001 ± 17 µg

The uncertainty has been calculated according to ISO 33405 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (6β,17β)-6,17-dihydroxy-17-methylandrosta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 3 July 2034, eight 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 periodically 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 under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D565. This material was prepared by sourced from an external supplier and certified for identity and purity by NMI Australia.

Intended use: This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times, each time with a minimum of 0.5 mL of a suitable organic solvent (e.g., methanol). This process will ensure the transfer of the stated mass per ampoule of anhydrous 6β-hydroxymethandienone. The mass of analyte contained in each ampoule has been determined based on the assigned purity of the bulk material and the concentration of that bulk material in the stock solution used during ampoule preparation.

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: At the recommended storage conditions this material has demonstrated stability for a period of five 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 HPLC with UV detection 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.
15 July 2026

This report supersedes any issued prior to 15 July 2026.

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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20A HT autosampler
	Column:	Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-8 min 30% B; 8-9 min 30-80% B; 9-14 min 80% B; 14-15 min 80-30% B, 15-22 min 30%B
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 248 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.002% (7 ampoules in duplicate, September 2018)
	Re-analysis	Mean = 99.8%, s = 0.003% (5 ampoules in duplicate, August 2019)
	Re-analysis	Mean = 99.8%, s = 0.001% (5 ampoules in duplicate, April 2022)
	Re-analysis	Mean = 99.8%, s = 0.001% (5 ampoules in duplicate, July 2026)

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

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection 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 elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	220 °C (1 min), 10 °C/min to 280 °C, 20 °C/min to 300 °C (1 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.04% (10 sub samples in duplicate, February 1999)
	Re-analysis:	Mean = 99.6%, s = 0.02% (7 sub samples in duplicate, June 2008)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (30:70 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 248 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, November 2010)
	Re-analysis:	Mean = 99.8%, s = 0.003% (7 sub samples in duplicate, September 2018)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue 0.3% mass fraction (June and November 2015)
Karl Fischer analysis:		Moisture content 0.16% mass fraction. (2 sub samples in AG solution, June 2008) Moisture content 0.14% mass fraction (2 sub samples in AK solution, July 2008) Moisture content 0.09% mass fraction (2 sub samples in AK solution, September 2018)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 2, 17 m x 0.22 mm ID x 0.11 μm
	Program:	140 °C (1 min), 8 °C/min to 250 °C, 30 °C/min to 300 °C (1 min)
	Injector:	280 °C
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	30/1
	Tris-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m x 0.25 mm ID x 0.22 μm
	Program:	180 °C (1 min), 12 °C/min to 310 °C (2 min)
	Injector:	250 °C
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the parent compound and *tris*-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 (14.9 min):	316 (M^+, 6), 298 (27), 283 (32), 265 (30), 225 (40), 107 (100) *m/z*
	Tris -TMS (7.4 min):	532 (M^+, 8), 519 (37), 518 (66), 517 (100), 229 (10), 73 (87) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate/chloroform (15:10:5) Single spot observed, R_f = 0.30. Visualisation with UV at 254 nm
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm-1, KBr pellet
	Peaks:	3497, 3390, 1661, 1620, 1452, 1401, 1048 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.95 (3H, s), 1.18 (3H, s), 1.44 (3H, s), 4.52 (1H, t), 6.13 (1H, d), 6.18 (1H, dd), 7.05 (1H, d) ppm
		Ethanol and dichloromethane estimated at 0.22% and 0.06% mass fraction respectively were observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker ARX-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 14.0, 20.3, 22.3, 23.3, 25.7, 30.8, 31.3, 38.8, 39.7, 43.7, 45.6, 49.8, 51.9, 73.7, 81.5, 125.6, 126.5, 157.6, 166.5, 186.8 ppm
Melting point:		227-229 °C
Microanalysis:	Found:	C = 75.9%; H = 9.0% (February 1999)
	Calculated:	C = 75.9%; H = 8.9% (Calculated for $C_{20}H_{28}O_3$)