



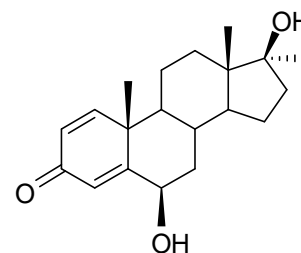
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D565: 6 β -Hydroxymethandienone

Report ID: D565.2019.01 (Ampouled 080623)

Chemical Formula: C₂₀H₂₈O₃

Molecular Weight: 316.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-000016	33526-41-9	989 ± 18 µg

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

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

Expiration of certification: The property values are valid till 22 August 2024, 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 and is intended for a single use to prepare a standard solution containing D565. Material was prepared by sourced from an external supplier, and certified for identity and purity by NMIA.

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

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 989 ± 18 µg of anhydrous 6 β -hydroxymethandienone.

Recommended storage: When not in use, this material should be stored at or below 4 °C 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: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 five 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.
4 September 2019

This report supersedes any issued prior to 4 September 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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20A HT autosampler	
	Column:	Alltima C-18, 5µm (4.6 mm × 150 mm)	
	Mobile Phase:	Acetonitrile/MilliQ water (30:70)	
	Flow Rate:	1.0 mL/min	
	Detector:	Shimadzu PDA SPD-M20A operating at 248 nm	
Relative mass fraction of the main component:			
	Initial analysis:	Mean = 99.8%, s = 0.002% (5 ampoules in duplicate, November 2010)	
	Re-analysis:	Mean = 99.8%, s = 0.002% (5 ampoules in duplicate, November 2011)	
	Re-analysis	Mean = 99.8%, s = 0.002% (5 ampoules in duplicate, September 2014)	
	Re-analysis	Mean = 99.8%, s = 0.002% (5 ampoules in duplicate, August 2019)	
GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	200 °C (1 min), 10 °C/min to 260 °C (3 min), 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 = 99.6%, s = 0.01% (7 ampoules in duplicate, June 2008)
		Re-analysis:	Mean = 99.7%, s = 0.09% (5 ampoules in duplicate, June 2009)

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 certified 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 ¹H NMR spectroscopy. The purity value is calculated as per Equation 1

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

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

Supporting evidence is provided by qualitative GC-FID analysis and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1 Capillary, 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 mass fraction 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)
	Mobile Phase:	Acetonitrile/MilliQ water (30:70) 0.05% TFA was present in both aqueous and organic phases.
	Flow Rate:	1.0 mL/min
	Detector:	Shimadzu PDA SPD-M20A operating at 248 nm
Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, November 2010)
Thermogravimetric analysis:	Volatiles content < 0.1% and non-volatile residue 0.3% mass fraction	
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)	

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	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
	<i>Tris</i> -Trimethylsilyl derivative:	
	Instrument:	HP6890/5973
	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
	Split ratio:	20/1
	The retention times of the parent material and its <i>tris</i> -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) <i>m/z</i>
	<i>Tris</i> -TMS (7.4 min):	532 (M ⁺ , 8), 519 (37), 518 (66), 517 (100), 229 (10), 73 (87) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate/chloroform (15:10:5) Single spot observed, R _f = 0.30 (5 sub samples)
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)
	Key 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 (77.16 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)