Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

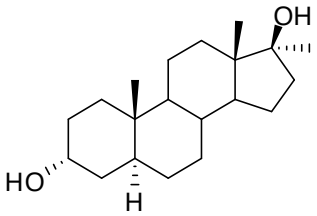
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D560: 17α-Methyl-5α-androstane-3α,17β-diol

Report ID: D560.2016.02 (Ampouled 101025)

Chemical Formula: $C_{20}H_{34}O_2$

Molecular Weight: 306.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-000005	**641-82-7**	**999 ± 16 μg**

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

IUPAC name: (3α,5α,17β)-17-Methylandrostane-3,17-diol.

Expiration of certification: The property values are valid till 3 August 2021, 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 supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D560. Material was sourced from an external supplier 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 999 ± 16 μg of anhydrous 17α-methyl-5α-androstane-3α,17β-diol. 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%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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 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.
10 February 2020

This report supersedes any issued prior to 10 February 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:

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 20 °C/min to 260 °C (5 min), 10 °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 = 99.9%, s = 0.002% (7 ampoules in duplicate, November 2010)	
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, September 2013)	
	Re-analysis:	Mean = 99.7%, s = 0.05% (5 ampoules in duplicate, August 2016)	

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 elemental microanalysis.

GC-FID:	Instrument:	HP 5890	
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C /min to 300 °C (6 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.8%, s = 0.004% (7 sub samples in duplicate, December 2004)	

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 20 °C/min to 260 °C (5 min), 10 °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 = 99.9%, s = 0.003% (5 sub samples in duplicate, March 2008)	
	Current analysis:	Mean = 99.9%, s = 0.014% (7 sub samples in duplicate, November 2010)	

Thermogravimetric analysis:	Volatile content < 0.1% and non volatile residue < 0.2 % mass fraction (June 1999 and May 2006)
Karl Fischer analysis:	Moisture content 0.17% mass fraction (March 2008) Moisture content 0.11% mass fraction (October 2010)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973		
Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 0.10 μm		
Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)		
Injector:	280 °C	Split inj (20/1)	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Scan range: 50-550 m/z	

Bis-TMS derivative:

Instrument:	Agilent 6890/5973		
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm		
Program:	170 °C (0.5 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)		
Injector:	280 °C	Split inj. (20/1)	Transfer line temp: 300 °C
Carrier:	Helium	Scan range: 50-550 m/z	

The retention times of the parent compound and *bis*-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 (5.85 min): 306 (M^+, 32), 291 (95), 230 (100), 215 (88), 165 (81), 107 (78) *m/z*
Bis -TMS (10.50 min): 450 (M^+, 2), 435 (17), 255 (7), 143 (100), 73 (34) *m/z*
The *bis*-silylated derivative of the synthetic material co-elutes with a derivatised comparison sample of 17α-methyl-5α-androstane-3α,17β-diol and the two materials produce matching mass spectra.

TLC:
Conditions: Kieselgel 60F_{254}. Ethyl acetate/dichloromethane (3:7)
Single spot observed, Rf = 0.36

IR:
Instrument: FT-IR, Biorad WIN FTS40
Range: 4000 - 400 cm^{-1}, KBr powder
Peaks: 3357, 1448, 1370, 1266, 1170, 1008 cm^{-1}

^{1}H NMR:
Instrument: Bruker Avance III-400
Field strength: 400 MHz Solvent: $CDCl_3$ (7.26 ppm)
Key spectral data: δ 0.79 (3H, s), 0.84 (3H, s), 1.20 (3H, s), 4.03 (1H, d, *J* =2.1 Hz) ppm

^{13}C NMR:
Instrument: Bruker Avance III-400
Field strength: 100 MHz Solvent: $CDCl_3$ (77.16 ppm)
Spectral data: δ 11.4, 14.1, 20.5, 23.4, 26.0, 28.6, 29.2, 31.8, 31.9, 32.4, 36.0, 36.3, 36.5, 39.1, 39.4, 45.7, 50.9, 54.5, 66.7, 81.9 ppm

Melting point: 183-186 °C

Microanalysis:
Found: C = 78.4%; H = 11.4% (June 2007)
Calculated: C = 78.4%; H = 11.2% (Calculated for $C_{20}H_{34}O_2$)