Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

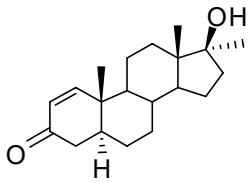
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D904: 17β-Hydroxy-17α-methyl-5α-androst-1-ene-3-one

Report ID: D904.2024.01 (Ampouled 110627)

Chemical Formula: $C_{20}H_{30}O_2$

Molecular Weight: 302.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
06-S-01	65-04-3	994 ± 18 μg

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

IUPAC name: (5α,17β)-17-Hydroxy-17-methylandrost-1-en-3-one

Expiration of certification: The property values are valid till 23 July 2034, 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 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 D904. This material was prepared by synthesis, 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. chloroform). This will transfer 994 ± 18 μg of anhydrous 17β-Hydroxy-17α-methyl-5α-androst-1-ene-3-one 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: This material has demonstrated stability over a minimum period of three 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.
14 August 2024

This report supersedes any issued prior to 14 August 2024.

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 7890 / Agilent 6890 / Varian CP-3800
	Column:	HP-1 or VF-1ms, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 20 °C/min to 250 °C (5 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 = 99.6%, s = 0.01% (7 ampoules in duplicate, June 2011)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, April 2014)
	Re-analysis:	Mean = 99.6%, s = 0.04% (5 ampoules in duplicate, March 2017)
	Re-analysis:	Mean = 99.7 %, s = 0.03% (5 ampoules in duplicate, February 2020)
	Re-analysis:	Mean = 99.6 %, s = 0.01% (5 ampoules in duplicate, July 2024)

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 from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID and Karl Fischer analysis. 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, thermogravimetric analysis and ^{1}H NMR.

GC-FID:	Instrument:	HP5890
	Column:	ZB-1, 28.5 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (10 sub samples in duplicate, February 2006)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.8%, s = 0.08% (5 sub samples in duplicate, February 2007)
GC-FID:	Instrument:	Varian 3800
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, January 2008)

GC-FID:	Instrument:	Varian 3800
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 20 °C/min to 250 °C (5 min), 40 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, February 2009)
Karl Fischer analysis:		Moisture content 0.2% mass fraction (January 2008) Moisture content < 0.8% mass fraction (February 2009)
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (February 2006 & February 2007 & February 2009)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP5890/5971A
	Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm
	Program:	220 °C (2 min), 10 °C/min to 300 °C (7 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m × 0.20 mm I.D. × 0.10 μm
	Program:	200 °C, 10 °C/min to 250 °C, 20 °C/min to 310 °C (2 min)
	Injector:	250 °C
	Split ratio:	12/1
	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 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 (8.23 min):	302 (M^+, 16), 245 (10), 160 (19), 149 (15), 134 (25), 122 (100), 107 (58), 91 (40), 81 (30), 71 (48) *m/z*
	Bis-TMS (5.1 min):	446 (M^+, 21), 431 (11), 356 (7), 246 (6), 194 (32), 179 (17), 163 (8), 143 (100), 73 (74) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/Chloroform (1/4) Single spot observed, R_f = 0.3 Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3517, 3336, 2928, 2868, 1664, 1597, 1471, 1450, 1439, 1368, 1272, 1163, 1084, 1066, 936, 782 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.88 (3H, s), 0.90-0.99 (2H , m), 1.02 (3H, s), 1.18-1.59 (10H , m), 1.21 (3H , s), 1.70-1.76 (2H, m), 1.78-1.83 (2H, m), 1.90 (1H, m), 2.21 (1H, dd, *J* = 4.0, 17.8 Hz), 2.36 (1H, dd, *J* = 14.2, 17.8 Hz), 5.84 (1H, dd, *J* = 0.7, 10.2 Hz), 7.14 (1H, d, *J* = 10.2 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Gyro-300
	Field strength:	75 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 13.0, 14.1, 20.9, 23.1, 25.8, 27.5, 31.0, 31.5, 36.5, 38.9, 39.0, 40.9, 44.4, 45.6, 50.0, 50.6, 81.5, 127.4, 158.3, 200.1 ppm
Melting point:		152-153 °C
Microanalysis:	Found:	C = 79.6 %; H = 9.8 % (March, 2006)
	Calculated:	C = 79.4 %; H = 10.0 % (Calculated for $C_{20}H_{30}O_2$)