Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

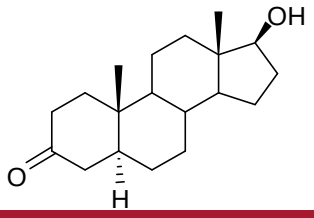
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D680: 5α-Dihydrotestosterone

Report ID: D680.2022.01 (Bottled 220622)

Chemical Formula: $C_{19}H_{30}O_2$

Molecular Weight: 290.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
01-S-03	521-18-6	93.9 ± 0.4%

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-Hydroxyandrostan-3-one.

Expiration of certification: The property values are valid till 22 June 2027, 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 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 crystals sourced from an external supplier, 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: 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 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.
1 February 2023

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:

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 qualitative elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890 or 7890
	Column:	HP-1 or HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 240 °C (4 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 as the free base:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (5 sub samples in duplicate, September 2010)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, September 2015)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, August 2017)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, July 2020)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, June 2022)
GC-FID:	Instrument:	HP 5890
	Column:	ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	240 °C (12 min)
	Injector:	250 °C
	Detector Temp:	325 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the free base:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (7 sub samples in duplicate, April 2000)
	Re-analysis:	Mean = 99.7%, s = 0.02% (7 sub samples in duplicate, September 2005)
Karl Fischer analysis:		Moisture content 3.0% mass fraction (September & October 2010) Moisture content 3.7% mass fraction (September 2011) Moisture content 5.4% mass fraction (September 2012) Moisture content 5.9% mass fraction (August 2015) Moisture content 5.8% mass fraction (August 2017) Moisture content 5.8% mass fraction (June 2020) Moisture content 5.8% mass fraction (June 2022)
Thermogravimetric analysis:		Volatiles content 3.0% and non-volatile residue < 0.2% mass fraction (September 2010)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Saturn 3400/2000 GC-MS Ion Trap
	Column:	J&W DB-17MS, 30 m x 0.25 mm I.D. x 0.17 μm
	Program:	220 °C (1 min), 10 °C /min to 280 °C (3 min)
	Injector:	250 °C
	Split ratio:	10/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	Saturn 3400/2000 GC-MS Ion Trap
	Column:	J&W DB-17MS, 30 m x 0.25 mm I.D. x 0.17 μm
	Program:	220 °C (1 min), 10 °C /min to 280 °C (3 min)
	Injector:	250 °C
	Split ratio:	10/1
	Transfer line temp:	280 °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 (8.5 min):	290 (M^+, 18), 275 (10), 272 (15), 257 (22), 231 (100), 213 (31) *m/z*
	Bis-TMS (6.1 min):	434 (M^+, 59), 419 (20), 405 (100), 377 (25), 343 (11), 73 (60) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20) Single spot observed, R_f = 0.4
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr disc
	Peaks:	3554, 3423, 1700, 1445, 1386, 1134, 1062 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-300
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.74 (3H, s), 1.00 (3H, s), 3.62 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	101 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 11.1, 11.4, 20.9, 23.3, 28.7, 30.4, 31.2, 35.3, 35.7, 36.6, 38.1, 38.5, 42.9, 44.6, 46.7, 50.8, 53.9, 81.7, 212.0 ppm
Melting point:		180-181 °C
Microanalysis:	Found:	C = 75.9%; H = 10.3% (October 2010)
	Calculated:	C = 78.6%; H = 10.4% (Calculated for $C_{19}H_{30}O_2$)