Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

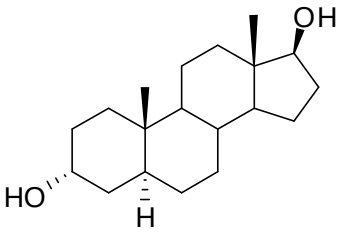
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D634b: 5a-Androstane-3a, 17β-diol

Report ID: D634b.2021.01 (Ampouled 180920)

Chemical Formula: $C_{19}H_{32}O_2$

Molecular Weight: 292.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
10-S-03	1852-53-5	997 ± 15 μg

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

IUPAC: (3α,5α,17β)-Androstane-3,17-diol

Expiration of certification: The property values are valid till 24 August 2024, i.e. three 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: 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 D634b. 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 997 ± 15 μg of anhydrous 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 three 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 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.
7 September 2021

This report supersedes any issued prior to 7 September 2021.

Characterisation Report:

GC-FID:	Instrument:	Varian CP-3800 / Agilent 7890A
	Column:	HP-5, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	230 °C (0.2 min), 5 °C/min to 265 °C, 30 °C/min to 280 °C (10 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *bis*-TMS derivative:	
	Initial analysis:	Mean = 99.5%, s = 0.007% (7 ampoules in duplicate, October 2018)
	Re-analysis:	Mean = 99.4%, s = 0.04% (5 sub samples in duplicate, August 2021)

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 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 quantitative NMR, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis. The purity value by qNMR was measured against a certified internal standard of potassium hydrogen maleate.

GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	230 °C (0.2 min), 5 °C/min to 265 °C, 30 °C/min to 280 °C (10 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *bis*-TMS derivative:	
	Initial analysis:	Mean = 99.5%, s = 0.03% (10 sub samples in duplicate, May 2010)
	Initial analysis:	Mean = 99.5%, s = 0.02% (5 sub samples in duplicate, May 2010)
	Re-analysis:	Mean = 99.5%, s = 0.02% (5 sub samples in duplicate, April 2015)
GC-FID:	Instrument:	Varian CP-3800
	Column:	VF-1MS, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (0.2 min), 7 °C/min to 230 °C, 20 °C/min to 280 °C (3 min), 10 °C/min to 265 °C (2 min), 30 °C/min to 300 °C (3min)
	Injector:	280 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *bis*-TMS derivative:	
	Initial analysis:	99.5%, s = 0.01% (5 sub samples in duplicate, May 2010)
Thermogravimetric analysis:		Volatile content 0.2% and non-volatile residue < 0.2% mass fraction (July 2010)
Karl Fischer analysis:		Moisture content 0.3% mass fraction (May 2010 and April 2015) Moisture content 6.1% (4 sub samples, March 2008 and 2 sub samples, October 2013) Moisture content 6.0% (2 sub samples, November 2016)
QNMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis:	Mean = 98.6%, s = 0.4% (5 sub samples in duplicate, May 2010)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	HP-1MS, 30 m x 0.32 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 20 °C/min to 240 °C (8 min), 30 °C/min to 300 °C (3 min)
Injector:	250 °C
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

Bis-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	HP-1MS, 30 m x 0.32 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 20 °C/min to 240 °C (8 min), 30 °C/min to 300 °C (3 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 *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 (7.3 min): 292 (M^+, 50), 277 (39), 274 (24), 259 (28), 233 (59), 215 (100), 165 (68), 148 (45), 133 (35), 121 (31), 107 (64), 92 (62), 79 (53), 67 (45), 55 (39), 41 (26) *m/z*

Bis-TMS (8.2 min): 436 (M^+, 20), 421 (18), 346 (30), 331 (32), 256 (55), 241 (100), 215 (53), 129 (93), 75 (93) *m/z*

HS-GC-MS:

Instrument:	Agilent 6890/5973/G1888
Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 µm
Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
Injector:	150 °C
Transfer line temp:	280 °C
Carrier:	Helium, 1.2 mL/min
Split ratio:	50/1
Solvents detected:	Chloroform

TLC:

Conditions: Kieselgel 60F_{254}. Dichloromethane/ethyl acetate (4/1)
Single spot observed, R_f = 0.29. Visualisation with vanillin stain

IR:

Instrument:	Biorad FTS300MX FT-IR
Range:	4000-400cm^{-1}, KBr powder
Peaks:	3392, 2967, 2922, 2848, 2681, 1442, 1362, 1266, 1166, 1133, 1048, 1005, 671 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III-400
Field strength:	400 MHz
Solvent:	MeOH-d_4 (3.31 ppm)
Spectral data:	δ 0.73 (3H, s), 0.72-0.79 (1H, m), 0.83 (3H, s), 0.86-1.08 (3H, m), 1.16-1.74 (16H, m), 1.82 (1H, ddd, *J* = 3.5, 3.5, 12.0 Hz), 1.96 (1H, m), 3.56 (1H, dd, *J* = 2.6, 8.6 Hz), 3.96 (1H, m) ppm

Methanol and chloroform were estimated at 0.1% mass fraction using ^{1}H NMR

^{13}C NMR:

Instrument:	Bruker Avance III-400
Field strength:	100 MHz
Solvent:	DMSO-d_6 (39.5 ppm)
Spectral data:	δ 11.1, 11.3, 20.0, 23.0, 28.2, 28.6, 29.8, 31.4, 32.0, 35.2, 35.68, 35.74, 36.6, 38.6, 42.5, 50.7, 54.2, 64.1, 80.1 ppm

Melting point: 223-224 °C

Microanalysis:

Found:	C = 77.5%; H = 11.2% (July 2010)
Calculated:	C = 78.0%; H = 11.0% (Calculated for $C_{19}H_{32}O_2$)