Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

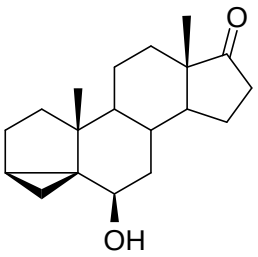
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S039: 3α-5-Cyclo-5α-androstane-6β-ol-17-one

Report ID: S039.2020.01 (Ampouled 170504)

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
16-S-10	663-39-8	995 ± 18 μg

The uncertainty is stated at the 95% coverage interval.

IUPAC name: (3β,5α,6β)-6-Hydroxy-3,5-cycloandrostan-17-one

Expiration of certification: The property values are valid till 21 August 2023, 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 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 S039. 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. acetonitrile). This will transfer 995 ± 18 μg of anhydrous 3α-5-cyclo-5α-androstan-6β-ol-17-one.

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.
4 September 2020

This report supersedes any issued prior to 4 September 2020.

Characterisation Report:

GC-FID:	Instrument:	Agilent 7890
	Column:	HP-5 or HP-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	100 °C (1 min), 30 °C/min to 235 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.03% (7 ampoules in duplicate, May 2017)
	Re-analysis:	Mean = 99.3%, s = 0.03% (5 ampoules in duplicate, October 2018)
	Re-analysis:	Mean = 99.3%, s = 0.03% (5 ampoules in duplicate, September 2019)
	Re-analysis:	Mean = 99.2%, s = 0.04% (5 ampoules in duplicate, August 2020)

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.

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

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

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

GC-FID:	Instrument:	Agilent 7890
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	100 °C (1 min), 30 °C/min to 235 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.05% (10 sub samples in duplicate, February 2017)
Thermogravimetric analysis:		The volatile content (e.g. organic solvents and/or water) could not be accurately determined by thermogravimetric analysis. Non-volatile residue < 0.2% mass fraction (January 2017)
Karl Fischer analysis:		Moisture content 0.2% mass fraction (February 2017)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 µm
	Program:	100 °C (1 min), 30 °C /min to 235 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention time of the parent compound is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (10.5 min):	288 (M^+, 8), 273 (79), 233 (100), 199 (11), 157 (11), 145 (17), 131 (23), 121 (25), 109 (21), 95 (32), 93 (27), 91 (41), 81 (12), 79(28), 77 (36), 67 (17), 55 (24) *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:	Diethyl ether
TLC:	Conditions:	Kieselgel 60F254. Diethyl ether/Hexane (1/1) Single spot observed, Rf = 0.16. Visualisation with vanillin
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3491, 2950, 2924, 2900, 2860, 2839, 1719, 1455, 1372, 1247, 1048, 1036, 1022, 1016, 926, 609, 583, 454 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	Benzene-d_6 (7.15 ppm)
	Spectral data:	δ 0.025 (1H, dd, *J* = 4.9, 8.1 Hz) , 0.33 (1H, t, *J* = 4.1 Hz), 0.58-0.63 (1H, m), 0.66 (3H, s), 0.71 (1H, m), 0.81-0.89 (3H, m), 1.00 (1H, s), 1.02 (3H, s), 1.08-1.26 (3H, m), 1.31-1.38 (2H, m), 1.44-1.51 (2H, m), 1.60 (1H, m), 1.75-1.82 (2H, m), 1.88 (1H, m), 1.95 (1H, dddd, *J* = 3.5, 11.2, 11.2, 11.2), 2.11 (1H, ddd, *J* = 0.9, 9.0, 19.0), 3.02, (1H, t, *J* = 2.6) ppm
		Diethyl ether estimated at 0.02% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	Benzene-d_6 (128.62 ppm)
	Spectral data:	δ 12.5, 14.3, 20.9, 22.4, 22.8, 24.8, 25.9, 30.3, 33.0, 34.0, 36.3, 36.8, 39.8, 43.8, 48.3, 48.7, 51.9, 73.7, 218.8 ppm
Melting point:		140-142 °C
Microanalysis:	Found:	C = 79.2%; H = 10.1% (February, 2017)
	Calculated:	C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)