Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

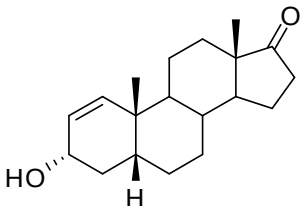
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S022: 5β-Androst-1-en-3α-ol-17-one

Report ID: S022.2024.01 (Ampouled 160721)

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

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
13-S-06	**33805-57-1**	**1000 ± 16 µg**

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

Synonyms: 3α-Hydroxy-5β-androst-1-en-17-one
3-Hydroxy-androst-1-en-17-one, (3α,5β)
3α-Hydroxy-5β-androst-1-en-17-one

Expiration of certification: The property values are valid till 2 April 2029, 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 and is intended for a single use to prepare a standard solution containing S022. This material was sourced from an external supplier 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. toluene). This will transfer 1000 ± 16 µg of anhydrous 5β-androst-1-en-3α-ol-3-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 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.
17 April 2024

This report supersedes any issued prior to 17 April 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:	Varian CP-3800
	Column:	TG-17, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 10 °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 *mono*-TMS derivative:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (7 ampoules in duplicate, July 2016)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, June 2017)
	Re-analysis:	Mean = 99.8%, s = 0.04% (5 ampoules in duplicate, June 2018)
	Re-analysis:	Mean = 99.8%, s = 0.05% (5 ampoules in duplicate, June 2019)
	Re-analysis:	Mean = 99.8%, s = 0.04% (5 ampoules in duplicate, April 2022)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, April 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 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:	Varian CP-3800
	Column:	TG-17, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 20 °C/min to 300 °C (3 min) [2013, 2015] or 180 °C (1 min), 30 °C/min to 240 °C (10 min), 10 °C/min to 280 °C (10 min) [2016]
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of main component as the *mono*-TMS derivative:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (10 sub samples in duplicate, August 2013)
	Re-analysis:	Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, July 2015)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, July2016)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP1, 30 m x 0.32 mm I.D x 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 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 main component as the *mono*-TMS derivative:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (10 sub samples in duplicate, August 2013)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, July 2014)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (July 2013, 2014, 2015 and 2016)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (July 2013). The volatile content (e.g. organic solvents and/or water) could not be determined because of degradation at elevated temperatures.

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)
Injector:	250 °C
Split ratio:	20/1
Transfer line temp:	280 °C
Carrier:	Helium

Bis-TMS derivative

Instrument:	Agilent 6890/5973
Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
Injector:	250 °C
Split ratio:	30/1
Transfer line temp:	280 °C
Carrier:	Helium

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 (9.8 min): 288 (M^+, 39), 270(55), 218 (75), 217 (21), 190 (33), 161 (41), 147 (38), 133 (22), 131 (23), 123 (35), 122 (54), 121 (26), 119 (35), 118 (39), 109 (57), 106 (73), 105 (71), 95 (48), 93 (68), 91 (100), 79 (61), 67 (42) *m/z*

Bis-TMS (8.4 min): 432 (M^+, 32), 417 (26), 327 (24), 290 (45), 275 (57), 195 (20), 182 (17), 181 (16), 181 (16), 169 (20), 75 (49), 73 (100) *m/z*

TLC:

Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (1/1) Single spot observed, R_f = 0.5. Visualization with vanillin.

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3491, 3017, 2969, 2921, 2850, 2826, 1730, 1723, 1450, 1373, 1260, 1207, 1055, 1028, 774 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance-400
Field strength:	400 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.86 (3H, s), 1.04 (3H, s), 1.07-1.71 (13H, m), 1.77-1.96 (4H, m), 2.06 (1H, dt, *J* = 9.0, 19.2 Hz), 2.42 (1H, ddd, *J* = 0.8, 9.0, 19.2 Hz), 4.33 (1H, m), 5.42 (1H, dt, *J* = 10.2, 1.7 Hz), 5.65 (1H, dd, *J* = 1.6, 10.1 Hz) ppm

Dichloromethane estimated at 0.06% mass fraction was observed in the ^{1}H NMR.

^{13}C NMR:

Instrument:	Bruker Avance-400
Field strength:	101 MHz
Solvent:	$CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.9, 21.2, 21.7, 21.9, 25.8, 27.8, 31.8, 35.0, 35.8, 35.9, 37.5, 40.1, 47.89, 47.94, 51.1, 69.3, 129.2, 140.0, 221.4 ppm

Melting point: 189-190 °C

Microanalysis:

Found:	C = 79.3%; H = 9.8 (July 2013)
Calculated:	C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)