Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

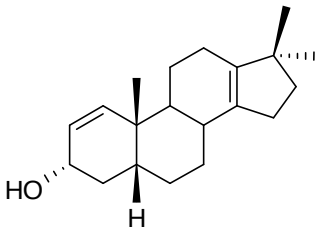
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D639: 18-Norandrostandien-3α-ol

Report ID: D639.2023.01 (Ampouled 111108)

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

Molecular Weight: 286.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
00-S-13	**186099-81-0**	**988 ± 38 μg**

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

IUPAC name: 17,17-Dimethyl-18-nor-5β-androsta-1, 13-dien-3α-ol

Expiration of certification: The property values are valid till 13 January 2028, 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 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 D639. 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 988 ± 38 μg of anhydrous 18-norandrostandien-3α-ol. 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.
13 January 2023

This report supersedes any issued prior to 13 January 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:

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.**

GC-FID:	Instrument:	Varian CP-3800 or Agilent 6890
	Column:	VF-1MS or HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (9 min), 20 °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 = 98.9%, s = 0.008% (7 ampoules in duplicate, November 2011)
	Re-analysis:	Mean = 98.8%, s = 0.003% (5 ampoules in duplicate, November 2012)
	Re-analysis:	Mean = 98.7%, s = 0.01% (5 ampoules in duplicate, September 2015)
	Re-analysis:	Mean = 98.7%, s = 0.03% (5 ampoules in duplicate, July 2018)
	Re-analysis:	Mean = 98.8%, s = 0.01% (5 ampoules in duplicate, January 2023)

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 qelemental microanalysis.

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.**

GC-FID:	Instrument:	Varian CP-3800
	Column:	VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (9 min), 20 °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 = 98.9%, s = 0.005% (5 sub samples in duplicate, November 2011)
GC-FID:	Instrument:	HP5890
	Column:	J&W DB-5MS or Zebron ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °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 = 98.2%, s = 0.07% (10 sub samples in duplicate, May 2000)
	Re-analysis:	Mean = 98.6%, s = 0.02% (5 sub samples in duplicate, April 2005)
HPLC:	Column:	Alltima C-18, 5 μm (4 mm x 150 mm)
	Mobile Phase:	Acetonitrile/MilliQ water (95:5 v/v)
	Flow rate:	0.8 mL/min Detector: ELSD and UV at 202 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean > 99% (2 sub samples, May 2000)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (October 2011)
Thermogravimetric analysis:		Volatiles content 0.25% and non-volatile residue < 0.2% mass fraction (April 2000) Volatiles content 0.2% and non-volatile residue < 0.2% mass fraction (April 2005) Volatiles content 0.3% and non-volatile residue < 0.2% mass fraction (November 2005)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP5890/5971
	Column:	BP-X5, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)
	Injector:	260 °C
	Split ratio:	30/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m x 0.25 mm ID x 0.22 μm
	Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times of the parent compound and 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 (6.9 min):	286 (M^+, 31), 271 (31), 253 (100), 185 (48), 163 (38) *m/z*
	TMS (4.3 min):	358 (M^+, 30), 343 (13), 268 (17), 253 (100), 216 (50) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (4:1) Single spot observed, R_f = 0.4 (3 sub samples)
IR:	Instrument:	Perkin-Elmer
	Range:	4000-400 cm^{-1}, Nujol mull
	Peaks:	3272, 1655, 1449, 1373, 1273, 1067, 1032, 938, 879, 714 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Key spectral data:	δ 0.94 (3H, s), 0.95 (3H, s), 0.99 (3H, s), 5.56 (1H, d), 5.79 (1H, d) ppm
	^{13}C NMR: Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 21.1, 22.1, 23.3, 26.2, 26.5, 26.7, 28.6, 29.8, 35.2, 37.0, 37.3, 39.5, 39.8, 45.0, 45.4, 69.2, 128.9, 135.5, 139.9, 141.3 ppm
Microanalysis:	Found:	C = 83.8%; H = 10.3% (April, 2000)
	Calculated:	C = 83.9%; H = 10.6% (Calculated for $C_{20}H_{30}O$)