Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

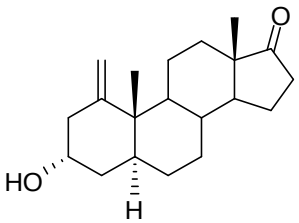
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D619: 1-Methylene-5α-androstan-3α-ol-17-one

Report ID: D619.2019.01 (Ampouled 160428)

Chemical Formula: $C_{20}H_{30}O_2$

Molecular Weight: 302.4 g/mol



Certified Value

Batch No.	CAS No.	Mass per ampoule
99-S-07	3398-66-1	947 ± 6 μg

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

IUPAC name: (3α,5α)-3-Hydroxy-1-methyleneandrostan-17-one (Metabolite of methenolone)

Expiration of certification: The property values are valid till 15 May 2024, 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 D619. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

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 947 ± 6 μg of anhydrous 1-methylene-5α-androstan-3α-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 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 five 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.
5 June 2019

This report supersedes any issued prior to 5 June 2019.

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	200 °C (24 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:		
	Initial analysis:	Mean = 97.4%, s = 0.015% (7 ampoules in duplicate, May 2016)	
GC-FID:	Instrument:	Varian CP-3800	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	200 °C (1 min), 5 °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 = 97.5%, s = 0.01% (5 ampoules in duplicate, May 2019)	

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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

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

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

Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Varian CP-3800	
	Column:	VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	210 °C (25 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:		
	Initial analysis:	Mean = 97.7%, s = 0.8% (7 sub samples in duplicate, February 2007)	
GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	200 °C (24 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:		
	Initial analysis:	Mean = 97.8%, s = 0.008% (7 sub samples in duplicate, May 2016)	
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler	
	Column:	Alltech C-18, 5 μm (4.6 mm x 150 mm)	
	Mobile Phase:	Acetonitrile/MilliQ water (55:45)	
	Flow rate:	1.0 mL/min	
	Detector:	Waters PDA 2998 operating at Max plot (190-400 nm) Water ELSD 2424	
	Relative peak area of the main component (PDA-Max plot):		
	Initial analysis:	Mean = 97.3%, s = 0.36% (7 sub samples in duplicate, September 2006)	
	Relative peak area of the main component (ELSD):		
	Initial analysis:	Mean = 99.8%, s = 0.04% (7 sub samples in duplicate, September 2006)	
Karl Fischer analysis:		Moisture content 2.8% mass fraction (October 2006) Moisture content 3.3% mass fraction (May 2016)	
Thermogravimetric analysis:		Volatile content < 1.8% and non-volatile residue < 0.2% mass fraction (January 2000 and September 2006)	

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	HP Ultra 2, 17 m × 0.22 mm I.D. × 0.11 μm
Program:	140 °C (1 min), 8 °C/min to 250 °C, 30 °C/min to 300 °C (3 min)
Injector:	280 °C Split ratio: splitless
Transfer line temp:	300 °C
Carrier:	Helium

Bis-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm
Program:	170 °C (0.5 min), 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

The retention times of the parent compound and *bis*-TMS derivative are reported along with the major peaks in the mass spectrum/spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.3 min): 302 (M+, 22), 284 (11), 269 (12), 256 (6), 217 (21), 199 (39), 91 (100) *m/z*

Bis-TMS (9.4 min): 446 (M+, 45), 431 (55), 341 (9), 169 (17), 73 (100) *m/z*

The *bis*-TMS derivative of the synthetic material co-elutes with a silylated comparison sample of 1-methylene 5α-androstan-3α-ol-17-one and the two materials produce matching mass spectra.

TLC:

Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate/chloroform (15:10:5) Single spot observed, R_f = 0.30. Visualisation with UV at 254 nm.

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3416, 3098, 1727, 1638, 1457, 1407, 1226, 1010 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Advance-300
Field strength:	300 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.91 (3H, s), 0.98 (3H, s), 4.03 (1H, m), 4.75 (1H), 4.82 (1H) ppm
	A steroid impurity believed to be the 3β diastereomer is observed in the ^{1}H NMR

^{13}C NMR:

Instrument:	Bruker Advance-300
Field strength:	75 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 12.6, 14.6, 21.7, 22.0, 28.4, 30.5, 31.9, 36.1, 36.4, 36.7, 41.5, 43.4, 43.6, 48.5, 49.5, 52.2, 67.6, 109.5, 151.4, 221.1 ppm

Melting point: 121-129 °C

Microanalysis:

Found:	C = 77.7%; H = 10.0% (April 2000)
Calculated:	C = 79.4%; H = 10.0% (Calculated for $C_{20}H_{30}O_2$)