

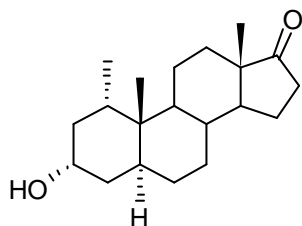


CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D557.2015.01 (Ampouled 111017)

This batch of ampoules was prepared from the bulk material on 17th October 2011.

Compound Name: 1α-Methyl-5α-androstan-3α-ol-17-one	Description: White crystals
Collection Number: D557	Batch Number: 98-002918
Chemical Formula: C ₂₀ H ₃₂ O ₂	Molecular Weight: 304.5
CAS Registry Number: 3398-67-2	Release Date: 20 May 2002
Structure:	Metabolite of mesterolone



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 D557. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. dichloromethane). This will transfer $1004 \pm 17 \mu\text{g}$ of anhydrous 1 α -methyl-5 α -androstan-3 α -ol-17-one. The uncertainty is stated at the 95% coverage interval.

Warning: This material is sensitive to methanol when dissolved and injected at elevated temperature (~ 250 °C) into a GC instrument.

GC-FID: Instrument: Agilent 7890
Column: HP-1 or HP-5, 30 m \times 0.32 mm I.D. \times 0.25 μm
Program: 180 °C (1 min), 10 °C/min to 230 °C (8 min), 30 °C/min to 300 °C (3 min)
Injector: 250 °C Detector Temp: 320 °C
Carrier: Helium Split ratio: 20/1
Relative peak area response of main component:
Initial analysis: Mean = 99.9%, s = 0.005% (7 ampoules in duplicate, October 2011)
Re-analysis: Mean = 99.8%, s = 0.01% (5 ampoules in duplicate, November 2012)
Re-analysis: Mean = 99.9%, s = 0.03% (5 ampoules in duplicate, September 2015)

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

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent and elemental microanalysis.

GC-FID:	Instrument:	Agilent 7890
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 230 °C (11 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Carrier:	Helium
		Detector Temp: 320 °C
		Split ratio: 20/1
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.007% (7 sub samples in duplicate, October 2011)
GC-FID:	Instrument:	Agilent 6890
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 240 °C, 20 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Carrier:	Helium
		Detector Temp: 320 °C
		Split ratio: 20/1
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.006% (10 sub samples in duplicate, June 2011)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 29.9 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
	Carrier:	Helium
		Detector Temp: 320 °C
		Split ratio: 20/1
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.8%, s = 0.025% (5 sub samples in duplicate, June 2006)
GC-FID:	Instrument:	Agilent 5890
	Column:	J&W DB-5MS, 30.0 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 240 °C, 20 °C/min to 280 °C (3 min)
	Injector:	250 °C
	Carrier:	Helium
		Detector Temp: 325 °C
		Split ratio: 20/1
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.1%, s = 0.11% (7 sub samples, February 1999)
Thermogravimetric analysis:	Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (February 1999, June 2006 and November 2011)	
Karl Fischer analysis:	Moisture content < 0.1% mass fraction (October 2011)	

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Saturn 3400/2000
	Column:	J&W DB-17 MS, 30 m x 0.25 mm I.D. x 0.17 μ m
	Program:	220 °C (1 min), 10 °C/min to 280 °C (3 min)
	Injector:	250 °C Transfer line temp: 280 °C
	Carrier:	Helium, 1.0 mL/min Split ratio: 25/1
	<i>Bis</i> -TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μ m
	Program:	170 °C (0.5 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
	Injector:	280 °C Transfer line temp: 300 °C
	Carrier:	Helium, 1.0 mL/min Split ratio: 20/1
	The retention times of the parent compound and <i>bis</i> -TMS derivative are reported along 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 (8.60 min): 304 (M^+ , 14), 286 (39), 271 (41), 218 (85), 199 (54), 175 (73), 161 (100), 119 (55), 91 (76) m/z	
	<i>Bis</i> -TMS (10.67 min): 448 (M^+ , 66), 433 (86), 343 (21), 253 (7), 169 (23), 73 (100) m/z	
	The <i>bis</i> -silylated derivative of the synthetic material co-elutes with a derivatised comparison sample of the metabolite from a urinary excretion study identified as silylated 1 α -methyl-5 α -androstan-3 α -ol-17-one. The two materials produce matching mass spectra.	
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	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:	None
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1} , KBr powder
	Peaks:	3499, 1726, 1452, 1407, 1170, 1009, 923, 846, 757 cm^{-1}
1H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.87 (3H, s), 0.88 (3H, s), 1.10 (3H, d), 4.06 (1H, br s) ppm
^{13}C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz Solvent: $CDCl_3$ (77.2 ppm)
	Spectral data:	δ 14.2, 14.3, 17.1, 19.8, 22.1, 28.9, 31.0, 31.9, 32.5, 35.5, 35.5, 36.1, 36.1, 36.8, 38.6, 48.2, 49.2, 52.0, 67.7, (220) ppm
Melting point:	158-159 °C	
Microanalysis:	Found: C = 78.9%; H = 10.8% (September, 1998) Calc: C = 78.9%; H = 10.6% (Calculated for $C_{20}H_{32}O_2$)	

Expiration of certification

The property values are valid till 22nd September 2020, 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.

The long-term stability of the compound in solution has not been examined.

This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

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.

Metrological Traceability

The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis.

Recommended storage

When not in use this material should be stored at or below 4 °C in a closed container in a dry, dark area.

Intended Use

For *in vitro* laboratory analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 30 September, 2015.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 30th September 2015.