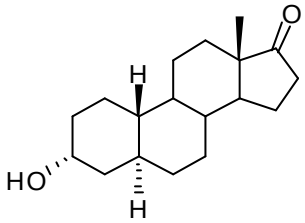
CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

Report ID: D555a.2017.01 (Ampouled 161020)
This batch of ampoules was prepared from the bulk material on 20th October 2016.

Compound Name: **19-Norandrosterone**
Collection Number: D555a
Chemical Formula: $C_{18}H_{28}O_2$
CAS Number: 1225-01-0
Structure:

Description: White crystals
Batch Number: 98-002917
Molecular Weight: 276.4
Release Date: 7th June 2000
Metabolite of nandrolone (19-nortestosterone)



Synonyms: 5α-Estran-3α-ol-17-one
3α-Hydroxy-5α-estran-17-one

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 D555a. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform). This will transfer 943 ± 23 µg of anhydrous 19-norandrosterone. The uncertainty is stated at the 95% coverage interval.

GC-FID:	Instrument:	Varian CP-3800, Agilent 7890A	
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 210 °C (8 min), 20 °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 = 99.6%, s = 0.005% (6 ampoules in duplicate, October 2016)	
	Re-analysis:	Mean = 99.6%, s = 0.008% (5 ampoules in duplicate, September 2017)	

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

Purity estimate 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 ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Varian CP-3800	
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 210 °C (8 min), 20 °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:		
	Re-analysis:	Mean = 99.6%, s = 0.009% (7 sub samples in duplicate, October 2013)	
GC-FID:	Instrument:	HP5890	
	Column:	J&W DB-1MS Capillary, 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 280 °C (3 min)	
	Injector:	280 °C	Detector Temp: 325 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	99.7%, s = 0.01% (10 sub samples in duplicate, October 1999)	
	Re-analysis:	99.7%, s = 0.02% (7 sub samples in duplicate, December 2004)	
	Re-analysis:	99.7%, s = 0.01% (5 sub samples in duplicate, March 2008)	
HPLC:	Peak area percentage of total > 99% (3 sub samples)		
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)	
	Mobile Phase:	Acetonitrile/water (60:40)	
	Flow Rate:	1.0 mL/min	
	Detector:	Refractive Index	
Thermogravimetric analysis:		Volatile content 5.1% and non-volatile residue < 0.2% mass fraction (June 2005)	
Karl Fischer analysis:		Moisture content 6.2% (2 sub samples, November 2006) Moisture content 6.1% (4 sub samples, March 2008 and 2 sub samples, October 2013) Moisture content 6.0% (2 sub samples, November 2016)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP6890/5973	
Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 0.10 μm	
Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)	
Injector:	280 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 15/1

Bis-TMS derivative

Instrument:	HP 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 inj. (15/1)	Transfer line temp: 300 °C
Carrier:	Helium	Scan range: 50-550 m/z

The retention times of the parent compound and its *bis*-TMS derivative are 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 (4.9 min): 276 (M^+, 100), 258 (21), 240 (16), 232 (35), 214 (18), 202 (48) 187 (33) m/z
Bis -TMS (8.4 min): 420 (M^+, 64), 405 (100), 315 (25), 225 (12), 169 (26), 73 (99) m/z

The *bis*-silylated derivative of the material co-elutes with a derivatised comparison sample of 19-norandrosterone and the two materials produce matching mass spectra.

TLC: Conditions: Kieselgel 60F_{254}. Hexane/tetrahydrofuran (5:2)
Single spot observed, R_f = 0.35 (5 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3510, 1731, 1450, 1105, 1013 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Advance-300	
Field strength:	300 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.80 (3H, s), 4.00 (1H, m) ppm	

^{13}C NMR:

Instrument:	Bruker Advance-300	
Field strength:	75 MHz	Solvent: $CDCl_3$ (77.16 ppm)
Spectral data:	δ 14.1, 22.0, 24.1, 25.2, 30.2, 31.9, 33.3, 33.8, 36.2, 36.3, 40.9, 41.1, 47.4, 48.2, 48.6, 51.0, 66.5, 222 ppm	

Melting point: 173-174 °C

Microanalysis: Found: C = 78.3%, H = 10.3%;
Calc: C = 78.2%, H = 10.2% for dried sample (September 1998)
Found: C = 73.8%, H = 10.4%;
Calc: C = 73.8%, H = 10.3% for 5.6% water mass fraction (June 2005)

Expiration of certification
The property values are valid till 20th September, 2022, 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 five 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 ºC 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: 26 September 2017

Characterisation data and property values specified in this report supersede those in all reports issued prior to 26th September 2017.