Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

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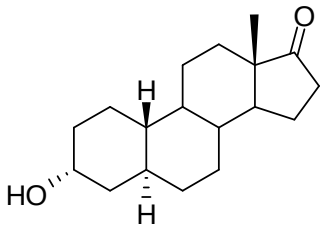
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D555a: 19-Norandrosterone

Report ID: D555a.2017.03 (Ampouled 161020)

Chemical Formula: $C_{18}H_{28}O_2$

Molecular Weight: 276.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
98-002917	**1225-01-0**	**943 ± 23 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-Hydroxyestran-17-one

Expiration of certification: The property values are valid till 20 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 sample bottles 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D555a. 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 943 ± 23 μg of anhydrous 19-norandrosterone. 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 five years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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 a 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.
31 January 2020.

This report supersedes any issued prior to 31 January 2020.

Characterisation Report:

GC-FID:	Instrument:	Varian CP-3800 / Agilent 7890A	
	Column:	HP-5, 30 m x 0.32 mm I.D. x 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 mass fraction of the 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.

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:	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 mass fraction of the 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 mass fraction of the 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)	

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, 222.0 ppm	

Melting point: 173-174 °C

Microanalysis:

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