



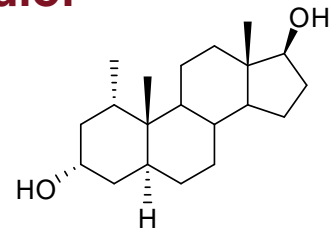
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D556: 1 $\alpha$ -Methyl-5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol

Report ID: D556.2016.02 (Ampouled 070430)

Chemical Formula: C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>

Molecular Weight: 306.5 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
98-002919	4349-94-4	996 $\pm$ 17 $\mu$ g

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

**IUPAC name:** (1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-1-Methylandrostane-3,17-diol.

**Expiration of certification:** The property values are valid till 9 March 2021, 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D556. The material was sourced from an external supplier 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 996  $\pm$  17  $\mu$ g of anhydrous 1 $\alpha$ -methyl-5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol. 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  $^{\circ}$ C 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 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 February 2020

This report supersedes any issued prior to 13 February 2020

**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.

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**Characterisation Report:**

GC-FID:	Instrument:	Agilent 6890N or Agilent 7890
	Column:	HP-1 Capillary, 30 m $\times$ 0.32 mm I.D. $\times$ 0.25 $\mu$ m
	Program:	180 $^{\circ}$ C (3 min), 30 $^{\circ}$ C/min to 240 $^{\circ}$ C (9 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Carrier:	Helium
		Detector Temp: 320 $^{\circ}$ C
		Split ratio: 20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.06% (7 sub samples, March 1999)
	Re-analysis:	Mean = 99.3%, s = 0.02% (5 ampoules in duplicate, May 2008)
	Re-analysis:	Mean = 99.4%, s = 0.01% (5 ampoules in duplicate, May 2011)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, March 2016)

**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

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

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

Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1 Capillary, 29.5 m $\times$ 0.32 mm I.D. $\times$ 0.25 $\mu$ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 250 $^{\circ}$ C (5 min), 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (5 min)
	Injector:	250 $^{\circ}$ C
	Carrier:	Helium
		Detector Temp: 320 $^{\circ}$ C
		Split ratio: 20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.06% (7 sub samples, March 1999)
	Re-analysis:	Mean = 99.3%, s = 0.02% (5 ampoules in duplicate, August 2006)
	Re-analysis:	Mean = 99.3%, s = 0.03% (7 sub samples in duplicate, April 2007)
Thermogravimetric analysis:	Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (June 1999 & March 2007)	
Karl Fischer analysis:	Moisture content 0.3% mass fraction (May 2007)	

**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 $\mu$ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 220 $^{\circ}$ C, 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	280 $^{\circ}$ C Transfer line temp: 300 $^{\circ}$ 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 $\mu$ m
	Program:	170 $^{\circ}$ C, 3 $^{\circ}$ C/min to 234 $^{\circ}$ C, 10 $^{\circ}$ C/min to 265 $^{\circ}$ C (3 min)
	Injector:	280 $^{\circ}$ C Transfer line temp: 300 $^{\circ}$ C
	Carrier:	Helium Split ratio: 20/1
	The retention times of the parent compound and the <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 (6.1 min):	306 ( $M^+$ , 47), 220 (100), 202 (69), 201 (53), 107 (63), 105 (50) <i>m/z</i>
	<i>Bis</i> -TMS (10.9 min):	450 ( $M^+$ , 24), 435 (20), 345 (28), 255 (24), 145 (77), 73 (100) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Hexane/ethyl acetate/chloroform (15:10:5) Single spot observed, $R_f$ = 0.23 (5 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 $\text{cm}^{-1}$ , KBr pellet
	Peaks:	3300, 1445, 1375, 1336, 1052, 1004, 919, 847, 758 $\text{cm}^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	$\text{CDCl}_3$ (7.26 ppm)
	Key spectral data:	$\delta$ 0.74 (3H, s), 0.86 (3H, s), 1.09 (3H, d, $J$ = 6.0 Hz), 3.61 (1H, t, $J$ = 9.0 Hz), 4.04 (1H, br s) ppm
<sup>13</sup> C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	$\text{CDCl}_3$ (77.2 ppm)
	Spectral data:	$\delta$ 11.6, 14.3, 17.1, 20.1, 23.7, 29.1, 30.6, 31.7, 32.5, 35.5, 35.9, 36.0, 36.8, 37.9, 38.4, 43.3, 49.1, 51.6, 67.3, 81.8 ppm
Melting point:		183-184.5 $^{\circ}$ C
Microanalysis:	Found:	C = 78.6%; H = 11.3% (April 2007)
	Calculated:	C = 78.4%; H = 11.2% (Calculated for $\text{C}_{20}\text{H}_{34}\text{O}_2$ )