



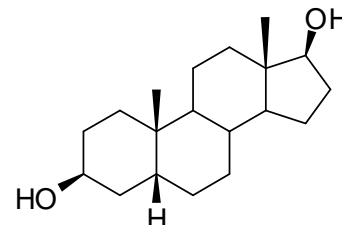
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D637: 5 $\beta$ -Androstane-3 $\beta$ , 17 $\beta$ -diol

Report ID: D637.2021.01 (Ampouled 100809)

Chemical Formula: C<sub>19</sub>H<sub>32</sub>O<sub>2</sub>

Molecular Weight: 292.5 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
98-001018	6038-31-9	927 $\pm$ 11 $\mu$ g

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

**IUPAC:** (3 $\beta$ ,5 $\beta$ ,17 $\beta$ )-Androstane-3,17-diol

**Expiration of certification:** The property values are valid till 18 February 2031, i.e. ten 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 D637. 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 927  $\pm$  11  $\mu$ g of anhydrous 5 $\beta$ -androstane-3 $\beta$ , 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%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

**Stability:** This material has demonstrated stability over a minimum period of ten 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 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.  
8 March 2021

This report supersedes any issued prior to 08 March 2021

**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:** Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA'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 document.

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## Characterisation Report:

GC-FID: Instrument: Varian 3800 or Agilent 7890  
 Column: VF-1, 30 m x 0.32 mm I.D. x 0.25 μm, or  
 HP-5, 30 m x 0.32 mm I.D. x 0.25 μm  
 Program: 220 °C (12 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 = 97.6%, s = 0.02% (7 ampoules in duplicate, August 2010)  
 Re-analysis: Mean = 97.4%, s = 0.02% (5 ampoules in duplicate, August 2011)  
 Re-analysis: Mean = 97.8%, s = 0.02% (5 ampoules in duplicate, August 2012)  
 Re-analysis: Mean = 97.7%, s = 0.03% (5 ampoules in duplicate, July 2013)  
 Re-analysis: Mean = 97.8%, s = 0.04% (5 ampoules in duplicate, May 2016)  
 Re-analysis: Mean = 97.8%, s = 0.02% (5 ampoules in duplicate, February 2021)

### 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 <sup>1</sup>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, 29.8 m x 0.32 mm x 0.25 μm  
 Program: 220 °C (10 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 = 97.8%, s = 0.13% (5 sub samples in duplicate, February 2007)

GC-FID: Instrument: Varian  
 Column: VF-1, 29.8 m x 0.32 mm x 0.25 μm  
 Program: 220 °C (10 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 = 98.1%, s = 0.16% (5 sub samples in duplicate, February 2008)  
 Re-analysis: Mean = 97.8%, s = 0.06% (5 sub samples in duplicate, February 2009)

Thermogravimetric analysis: Volatiles content 4.9% and non-volatile residue < 0.2% mass fraction (February 2007)  
 Volatiles content 5.3% and non-volatile residue < 0.2% mass fraction (January 2008)  
 Volatiles content 5.9% and non-volatile residue < 0.2% mass fraction (January 2009)  
 Volatiles content 5.2% and non-volatile residue < 0.2% mass fraction (August 2010)

Karl Fischer analysis: Moisture content 2.6% mass fraction (January 2008)  
 Moisture content 2.8% mass fraction (January 2009)

### Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Columns:	ZB-5 MS, 30 m x 0.25 mm I.D. x 0.25 $\mu$ m
	Program:	100 $^{\circ}$ C, 15 $^{\circ}$ C/min to 230 $^{\circ}$ C, 8 $^{\circ}$ C/min to 310 $^{\circ}$ C
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	300 $^{\circ}$ C
	Split ratio:	30/1
	The retention time of the parent compound is reported with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios (in brackets) as a percentage relative to the base peak.	
	9.3 min:	292 ( $M^+$ , 9), 274 (100), 259 (33), 241 (30), 233 (30), 215 (77) $m/z$
TLC:	Conditions:	Kieselgel 60F254. Chloroform/ethyl acetate (80:20) Single spot observed, $R_f$ = 0.13 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 $cm^{-1}$ , KBr powder
	Peaks:	3387, 1445, 1314, 1277, 1105, 1058, 1032, 944 $cm^{-1}$
$^1H$ NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3/DMSO-d_6$ (7.26 ppm/2.5 ppm)
	Key spectral data:	$\delta$ 0.55 (3H, s), 0.79 (3H, s), 3.42 (1H, t), 3.89 (1H, br) ppm $^1H$ NMR run in $d_4$ -methanol shows the presence of hexane and ethyl acetate in quantities of 3.0% and 0.5% mass fractions respectively.
$^{13}C$ NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	$CDCl_3/DMSO-d_6$ (77.16 ppm/39.52 ppm)
	Spectral data:	$\delta$ 11.0, 20.4, 23.2, 23.7, 25.7, 26.4, 27.6, 29.8, 30.2, 33.3, 35.0, 35.5, 36.3, 36.9, 39.8, 42.9, 51.0, 66.2, 81.3 ppm
Microanalysis:	Found:	C = 76.5%; H = 11.4% (May 2000)
	Calculated:	C = 78.0%; H = 11.0% (Calculated for $C_{19}H_{32}O_2$ )