Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

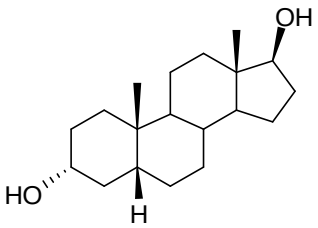
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D636: 5β-Androstane-3α,17β-diol

Report ID: D636.2018.03 (Ampouled 181108)

Chemical Formula: $C_{19}H_{32}O_2$

Molecular Weight: 292.46 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
98-001017	**1851-23-6**	**1000 ± 18 µg**

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

IUPAC: (3α,5β,17β)-Androstane-3,17-diol

Expiration of certification: The property values are valid till 14 December 2021, i.e. three years from the date of 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 D636. This 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. methanol). This will transfer 1000 ± 18 µg of anhydrous 5β-androstane-3α, 17β-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 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%. 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: In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years. 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.
31 January 2020

This report supersedes any issued prior to 31 January 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.

Characterisation Report:

GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	230 °C (1 min), 5 °C/min to 265 °C, 20 °C/min to 280 °C (6 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (7 ampoules in duplicate, December 2018)

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:	Agilent 6890N or Varian CP-3800
	Column:	30 m x 0.32 mm I.D. x 0.25 µm
	Program:	215 °C (20 min), 20 °C/min to 300 °C (5 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% (10 sub samples in duplicate, 1999)
	Re-analysis:	Mean = 99.8%, s = 0.1% (10 sub samples in duplicate, February 2005) (HP-1)
	Re-analysis:	Mean = 99.6%, s = 0.1% (10 sub samples in duplicate, February 2010) (HP-1)
	Initial analysis:	Mean = 99.8%, s = 0.1% (10 sub samples in duplicate, February 2010) (HP-5)
	Initial analysis:	Mean = 99.5%, s = 0.1% (10 sub samples in duplicate, February 2010) (VF-1)
	Initial analysis:	Mean = 99.9%, s = 0.04% (10 sub samples in duplicate, February 2010) (VF-1)
	Initial analysis:	Mean = 99.8%, s = 0.1% (10 sub samples in duplicate, February 2010) (HP-1)
GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	230 °C (0.2 min), 5 °C/min to 265 °C (2 min), 30 °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 as the *bis*-TMS derivative:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, April 2010)
	Re-analysis:	Mean = 99.9%, s = 0.02% (5 sub samples in duplicate, April 2015)
GC-FID:	Instrument:	Agilent 5890
	Column:	ZB-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	230 °C (0.2 min), 5 °C/min to 265 °C (2 min), 30 °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 as the *bis*-TMS derivative:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (10 sub samples in duplicate, April 2010)
Thermogravimetric analysis:		The volatile content (e.g. organic solvents and/or water) could not be analysed accurately because of the inherent volatility of the material. Non volatile residue < 0.2 % mass fraction (February 2010)
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (February 2010) Moisture content < 0.1% mass fraction (April 2015)

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 μm
	Program:	100 °C, 15 °C/min to 230 °C, then 8 °C/min to 310 °C
	Injector:	250 °C
	Transfer line temp:	300 °C
	Split ratio:	20/1
	The retention time of the parent material is 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.	
	9.4 min:	292 (M^+, 6), 274 (65), 256 (52), 241 (47), 215 (100) *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3343, 2924, 2864, 1469, 1452, 1367, 1276, 1172, 1056, 1036 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$/DMSO-d_6 (7.26 ppm/2.5 ppm)
	Key spectral data:	δ 0.55 (3H, s), 0.76 (3H, s), 3.42 (1H, m), 3.44 (1H, t) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4/DMSO-d_6 (49.0 ppm/39.52 ppm)
	Spectral data:	δ 11.1, 20.3, 23.2, 23.3, 26.1, 27.1, 30.0, 30.4, 34.6, 35.5, 36.0, 36.4, 37.2, 40.8, 42.2, 43.0, 51.2, 70.5, 80.7 ppm
Microanalysis:	Found:	C = 78.1%; H = 11.1% (May 2000)
	Calculated:	C = 78.0%; H = 11.0% (Calculated for $C_{19}H_{32}O_2$)