



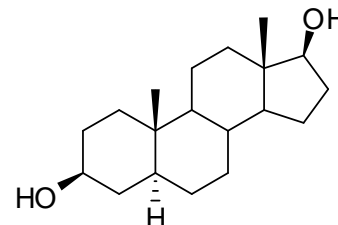
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D635: 5 α -Androstane-3 β , 17 β -diol

Report ID: D635.2019.02 (Ampouled 100816)

Chemical Formula: C₁₉H₃₂O₂

Molecular Weight: 292.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
98-001020	571-20-0	946 ± 8 µg

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

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

Expiration of certification: The property values are valid till 23 July 2024, 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 D635. 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 946 ± 8 µg of anhydrous 5 α -androstane-3 β , 17 β -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 °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 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 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: 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.

Characterisation Report:

GC-FID: Instrument: Varian CP-3800
 Column: DB-17, 29.6 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 5 $^{\circ}$ C/min to 280 $^{\circ}$ C (10 min)
 Injector: 200 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

Relative mass fraction of the main component:

Initial analysis: Mean = 99.8%, s = 0.01% (5 ampoules in duplicate, July 2019)

GC-FID: Instrument: Varian CP-3800
 Column: HP-1MS Capillary, 30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 150 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 240 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
 Injector: 250 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

Relative mass fraction of the main component:

Initial analysis: Mean = 99.1%, s = 0.02% (7 ampoules in duplicate, August 2010)

Re-analysis: Mean = 98.9%, s = 0.09% (5 ampoules in duplicate, August 2011)

Re-analysis: Mean = 99.1%, s = 0.02% (5 ampoules in duplicate, June 2014)

Re-analysis: Mean = 99.1%, s = 0.01% (5 ampoules in duplicate, April 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, 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_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by thermogravimetric analysis and elemental microanalysis.

GC-FID: Instrument: Varian CP-3800
 Column: DB-17, 29.6 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 5 $^{\circ}$ C/min to 280 $^{\circ}$ C (10 min)
 Injector: 200 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

Relative mass fraction of the main component:

Initial analysis: Mean = 99.8%, s = 0.02% (16 sub samples in duplicate, December 2018)

GC-FID: Instrument: HP5890
 Column: J&W DB-5MS Capillary, 30 m \times 0.25 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 240 $^{\circ}$ C, 20 $^{\circ}$ C/min to 280 $^{\circ}$ C (3 min)
 Injector: 250 $^{\circ}$ C Detector Temp: 325 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

Relative mass fraction of the main component:

Initial analysis: Mean > 99.9% (7 sub samples in duplicate, May 1999)

Re-analysis: Mean = 99.5%, s = 0.04% (5 sub samples in duplicate, May 2005)

GC-FID: Instrument: Varian CP3800
 Column: HP-1MS Capillary, 29.5 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 150 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 240 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
 Injector: 250 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

Relative peak area response of the main component:

Initial analysis: Mean = 99.2%, s = 0.04% (5 sub samples in duplicate, June 2010)

Thermogravimetric analysis: Volatiles content 2.3% and non-volatile residue < 0.2% mass fraction (April 2005)

Karl Fischer analysis: Moisture content 5.8% mass fraction (August 2006)
 Moisture content 5.4% mass fraction (May 2010)

Spectroscopic and other characterisation data

GC-MS:	Parent compound and <i>bis</i> -trimethylsilyl derivative: Instrument: Varian Saturn 3400/2000 Ion Trap GC-MS Column: J&W DB-17MS, 30 m \times 0.25 mm I.D. \times 0.17 μ m Program: 220 $^{\circ}$ C (1 min), 10 $^{\circ}$ C /min to 280 $^{\circ}$ C (3 min) Injector: 250 $^{\circ}$ C Transfer line temp: 280 $^{\circ}$ C Carrier: Helium, 1.0 mL/min Split ratio: 10/1 The retention times of the parent compound and <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 (7.9 min): 292 (M+, 22), 277 (8), 274 (15), 259 (23), 241 (42), 215 (100) <i>m/z</i> <i>Bis</i> -TMS (5.8 min): 421 (M+-CH ₃ , 31), 379 (6), 346 (17), 331 (15), 255 (33), 241 (100) <i>m/z</i> The <i>bis</i> -TMS derivative of D635 co-eluted and gave identical mass spectra to a comparison sample of silylated 5 α -androstane-3 β ,17 β -diol under these conditions.
	Instrument: HP6890/5973 Columns: HP Ultra 1, 17 m \times 0.22 mm ID \times 0.11 μ 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: 15/1 <i>Bis</i> -TMS (10.3 min): 436 (M+, 17), 421 (53), 346 (29), 331 (18), 256 (17), 241 (41), 75 (100) <i>m/z</i>
TLC:	Conditions: Kieselgel 60F254. Chloroform/ethyl acetate (80:20) Single spot observed, R _f = 0.23 (3 sub samples)
IR:	Instrument: FT-IR, Biorad WIN FTS40 Range: 4000-400 cm^{-1} , KBr powder Peaks: 3481, 3231, 1469, 1445, 1324, 1277, 1136, 1057, 1028 cm^{-1}
¹ H NMR:	Instrument: Bruker DMX-500 Field strength: 500 MHz Solvent: MeOH- <i>d</i> ₄ (3.31 ppm) Key spectral data: δ 0.72 (3H, s), 0.84 (3H, s), 3.29 (1H, t), 3.55 (1H, m) ppm Methanol estimated at 0.1% mass fraction was observed in the ¹ H NMR
¹³ C NMR:	Instrument: Bruker DMX-500 Field strength: 126 MHz Solvent: MeOH- <i>d</i> ₄ (49.0 ppm) Spectral data: δ 11.2, 12.5, 19.7, 22.2, 27.5, 29.0, 30.5, 34.4, 35.0, 41.8, 43.6, 49.9, 53.3, 42.5, 44.8, 50.9, 54.5, 70.5, 81.2 ppm
Melting point:	163-166 $^{\circ}$ C
Microanalysis:	Found: C = 74.0%; H = 11.2% (August 2006) Calculated: C = 78.0%; H = 11.0% (Calculated for C ₁₉ H ₃₂ O ₂)