Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

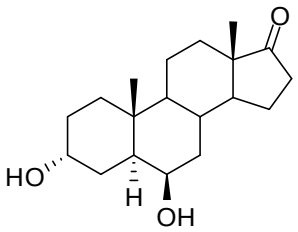
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D886b: 3α,6β-Dihydroxy-5α-androstan-17-one

Report ID: D886b.2019.01 (Ampouled 160908)

Chemical Formula: $C_{19}H_{30}O_3$

Molecular Weight: 306.4 g/mol




## Certified value

| Batch No. | CAS No. | Mass per ampoule |
|---|---|---|
| **15-S-11** | **152886-16-3** | **975 ± 22 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,6β)-3,6-Dihydroxyandrostan-17-one.

**Expiration of certification:** The property values are valid till 1 July 2022, i.e. three 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 and is intended for a single use to prepare a standard solution containing D886b. 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. DCM). This will transfer 975 ± 22 μg of anhydrous 3α,6β-Dihydroxy-5α-androstan-17-one.

**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 three 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.
11 July 2019

This report supersedes any issued prior to 11 July 2019.

**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: | Agilent 7890 |
| | Column: | HP-5, 30 m × 0.32 mm I.D. × 0.25 μm |
| | Program: | 190°C (1 min), 10 °C/min to 240 °C (20 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 = 99.1%, s = 0.06% (5 ampoules in duplicate, July 2019) |
| GC-FID: | Instrument: | Varian CP-3800 |
| | Column: | HP-1, 30 m × 0.32 mm I.D. × 0.25 μm |
| | Program: | 180 °C (1 min), 30 °C/min to 230 °C (12 min), 25 °C/min to 300 °C (3 min) |
| | Injector: | 250 °C |
| | Detector Temp: | 320 °C |
| | Carrier: | Helium |
| | Split ratio: | 20/1 |
| | Relative mass fraction of main component as the *Bis*-TMS and the *Tris*-TMS derivative: | |
| | Initial analysis: | Mean = 99.5%, s = 0.01% (7 ampoules in duplicate, September 2016) |
| | Reanalysis: | Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, August 2017) |
| | Re-analysis: | Mean = 98.9%, s = 0.03% (5 ampoules in duplicate, July 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 qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

| | | |
|---|---|---|
| GC-FID: | Instrument: | Varian CP-3800 |
| | Column: | HP-1, 30 m × 0.32 mm I.D. × 0.25 μm |
| | Program: | 180 °C (1 min), 30 °C/min to 230 °C (12 min), 25 °C/min to 300 °C (3 min) |
| | Injector: | 250 °C |
| | Detector Temp: | 320 °C |
| | Carrier: | Helium |
| | Split ratio: | 20/1 |
| | Relative mass fraction of main component as the *Bis*-TMS and the *Tris*-TMS derivative: | |
| | Initial analysis: | Mean = 99.9%, s = 0.006% (10 sub samples in duplicate, June 2016) |
| Thermogravimetric analysis: | | Volatile content 0.6% and non volatile residue < 0.2% mass fraction (June 2016) |
| Karl Fischer analysis: | | Moisture content 0.2% mass fraction (September 2015)<br>Moisture content 1.0% mass fraction (August 2016) |

## Spectroscopic and other characterisation data

| | | |
|---|---|---|
| GC-MS: | Parent compound: | |
| | Instrument: | Agilent 6890 |
| | Column: | TG-1MS, 30 m × 0.25 mm I.D. × 0.25 µm |
| | Program: | 220 °C (1 min), 10 °C/min to 280 °C (2 min), 10 °C/min to 300 °C (3 min) |
| | Injector: | 250 °C |
| | Transfer line temp: | 280 °C |
| | Carrier: | Helium, 1.0 mL/min |
| | Split ratio: | 100/1 |
| | *Tris*-TMS derivative: | |
| | Instrument: | Agilent 6890/5973 |
| | Column: | TG-1MS, 30 m × 0.25 mm I.D. × 0.25 µm |
| | Program: | 220 °C (1 min), 10 °C/min to 280 °C (2 min), 10 °C/min to 300 °C (3 min) |
| | Injector: | 250 °C |
| | Transfer line temp: | 280 °C |
| | Carrier: | Helium, 1.0 mL/min |
| | Split ratio: | 100/1 |
| | The retention times of the parent compound and *tris*-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): | 306 ($M^+$, 86), 288 (23), 270 (43), 255 (33), 230 (28), 213 (31), 150 (40), 147 (31), 145 (28), 134 (28), 123 (48), 119 (34), 107 (58), 95 (100), 91 (45), 81 (44), 79 (61), 67 (62), 55 (66) *m/z* |
| | *Tris*-TMS (7.4 min): | 522 (88), 507 (54), 417 (72), 327 (92), 237 (14), 221 (14), 195 (21), 182 (33), 169 (50), 147 (19), 129 (23), 105 (17), 91 (14), 73 (100) *m/z* |
| HS-GC-MS: | Instrument: | Agilent 6890/5973/G1888 |
| | Column: | DB-624, 30 m x 0.25 mm I.D. x 1.4 µm |
| | Program: | 50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min) |
| | Injector: | 150 °C |
| | Transfer line temp: | 280 °C |
| | Carrier: | Helium, 1.2 mL/min |
| | Split ratio: | 50/1 |
| | Solvents detected: | Ethyl acetate |
| TLC: | Conditions: | Kieselgel 60$F_{254}$. 100% ethyl acetate<br>Single spot observed, $R_f$ = 0.28<br>Visualisation with vanillin, $H_2SO_4$ spray |
| IR: | Instrument: | Bruker Alpha FT-IR |
| | Range: | 4000-400 $cm^{-1}$, neat |
| | Peaks: | 3571, 3434, 2945, 2922, 2856, 1715, 1613, 1434, 1368, 1244, 1024 $cm^{-1}$ |
| $^1$H NMR: | Instrument: | Bruker Avance III-500 |
| | Field strength: | 500 MHz |
| | Solvent: | MeOH-$d_4$ (3.31 ppm) |
| | Spectral data: | δ 0.86 (1H, m), 0.91 (3H, s), 1.04 (3H, s), 1.21-1.45 (7H, m), 1.54-1.73 (5H, m), 1.77 (1H, dt, *J* = 12.7, 3.6 Hz), 1.92-2.12 (5H, m), 2.45 (1H, dd, *J* = 8.5, 19.2 Hz), 3.71 (1H, dd, *J* = 2.6, 5.0 Hz), 4.07 (1H, quintet, *J* = 2.7 Hz) ppm |
| | | Ethyl acetate estimated at 0.5% mass fraction was observed in the $^1$H NMR |
| $^{13}$C NMR: | Instrument: | Bruker Avance III-500 |
| | Field strength: | 126 MHz |
| | Solvent: | MeOH-$d_4$ |
| | Spectral data: | δ 14.2, 15.3, 21.0, 22.7, 29.6, 31.3, 32.8, 34.1, 35.2, 36.7, 37.4, 39.5, 43.3, 49.2, 52.5, 55.9, 67.3, 72.4, 224.2 ppm |
| Melting point: | | 209-210 °C |
| Microanalysis: | Found: | C = 74.5%; H = 10.0%; (September 2015) |
| | Calculated: | C = 74.5%; H = 9.9%; (Calculated for $C_{19}H_{30}O_3$) |