



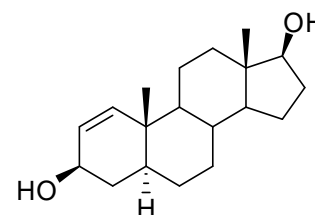
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D871b: 5 α -Androst-1-ene-3 β ,17 β -diol

Report ID: D871b.2017.02 (Ampouled 130805)

Chemical Formula: C₁₉H₃₀O₂

Molecular Weight: 290.4 g/mol



Certified value

| Batch No. | CAS No. | Mass per ampoule |
|-----------|-----------|------------------|
| 12-S-02 | 5323-27-3 | 913 μ g |

IUPAC name: (3 β ,5 α ,17 β)-Androst-1-ene-3,17-diol.

Expiration of certification: The property values are valid till 25 May 2022, 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. In the event a product fails the stability trial, notification will be sent to all impacted customers.

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 D871b. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This reference material is recommended for qualitative analysis only.

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 approximately 913 μ g of anhydrous 5 α -androst-1-ene-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 $^{\circ}$ C in a closed container in a dry, dark area.

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.
10 February 2020

This report supersedes any issued prior to 10 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.

Characterisation Report:

GC-FID: Instrument: Agilent 6890
 Column: HP-1, 30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 220 $^{\circ}$ C (10 min), 20 $^{\circ}$ 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 of the main component:
 Initial analysis: Mean = 96.4%, s = 0.08% (7 ampoules in duplicate, August 2013)
 Re-analysis: Mean = 96.4%, s = 0.09% (5 ampoules in duplicate, July 2014)
 Re-analysis: Mean = 96.3%, s = 0.06% (5 ampoules in duplicate, May 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, 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_{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.

Warning: The parent compound and *bis*-TMS derivatise are sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 160-250 $^{\circ}$ C) into a GC instrument.

GC-FID: Instrument: Varian CP-3800
 Column: HP-5, 30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 30 $^{\circ}$ C/min to 250 $^{\circ}$ C (10 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 of the main component:
 Initial analysis: Mean = 96.6%, s = 0.1% (10 sub samples in duplicate, September 2012)

GC-FID: Instrument: Agilent 6890
 Column: HP-1, 30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 220 $^{\circ}$ C (10 min), 20 $^{\circ}$ 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 of the main component:
 Initial analysis: Mean = 97.0%, s = 0.1% (10 sub samples in duplicate, September 2012)

Thermogravimetric analysis: Volatile content 5.3% and non volatile residue < 0.2% mass fraction (September 2012)

Karl Fischer analysis: Moisture content 3.3% mass fraction (September 2012)

Spectroscopic and other characterisation data

GC-MS: Parent compound:
Instrument: Agilent 6890/5973
Column: TG1-MS, 30 m x 0.25 mm I.D. x 0.25 μ m
Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (2 min)
Injector: 250 $^{\circ}$ C
Transfer line temp: 280 $^{\circ}$ C
Carrier: Helium, 1.0 mL/min
Split ratio: 20/1

Bis-TMS derivative:
Instrument: Agilent 6890/5973
Column: TG1-MS, 30 m x 0.25 mm I.D. x 0.25 μ m
Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (2 min)
Injector: 250 $^{\circ}$ C
Transfer line temp: 280 $^{\circ}$ C
Carrier: Helium, 1.0 mL/min
Split ratio: 20/1

The retention times of the parent compound and *bis*-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 (10.2 min): 290 (M^+ , 39), 272 (29), 220 (69), 202 (37), 187 (35), 161 (45), 147 (36), 135 (25), 118 (36), 105 (72), 91 (100), 79 (60), 55 (42) *m/z*

Bis-TMS (11.4 min): 434 (M^+ , 51), 405 (25), 202 (19), 143 (100), 129 (39), 127 (34), 105 (24), 75 (75), 73 (90) *m/z*

HS-GC-MS: Instrument: Agilent 6890/5973/G1888
Column: DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
Program: 50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
Injector: 150 $^{\circ}$ C
Transfer line temp: 280 $^{\circ}$ C
Carrier: Helium, 1.2 mL/min
Split ratio: 50/1
Solvents detected: Ethyl acetate, hexane, chloroform

TLC: Conditions: Kieselgel 60F₂₅₄. Chloroform/ethyl acetate (2/1)
Single spot observed, R_f = 0.4. Visualisation with vanillin

IR: Instrument: Biorad FTS3000MX FT-IR
Range: 4000-400 cm^{-1} , KBr powder
Peaks: 3463, 3392, 3221, 3021, 2929, 2845, 1645, 1446, 1324, 1126, 1067, 1026, 976, 871, 753, 605 cm^{-1}

¹H NMR: Instrument: Bruker Avance-400
Field strength: 400 MHz
Solvent: CDCl₃ (7.26 ppm)
Spectral data: δ 0.74 (3H, s), 0.79-1.08 (4H, m), 0.91 (3H, s), 1.19-1.61 (11H, m), 1.67-1.84 (4H, m), 2.09 (1H, m), 3.62 (9H, bm), 4.29 (1H, bs), 5.48 (1H, ddd, J = 1.7, 1.7, 10.2 Hz), 5.91 (1H, dd, J = 1.8, 10.2 Hz) ppm

Ethyl acetate estimated at 0.5% mass fraction was observed in the ¹H NMR, while chloroform and hexane could not be quantified due to overlapping peaks.

¹³C NMR: Instrument: Bruker Avance-400
Field strength: 101 MHz
Solvent: CDCl₃ (77.0 ppm)
Spectral data: δ 11.2, 15.7, 20.8, 23.3, 27.9, 30.5, 31.4, 35.6, 35.8, 36.6, 38.1, 43.1, 43.5, 51.0, 51.5, 68.8, 81.8, 128.7, 137.9 ppm

Melting point: 159-162 $^{\circ}$ C

Microanalysis: Found: C = 76.8%; H = 10.6% (September, 2012)
Calculated: C = 78.6%; H = 10.4% (Calculated for C₁₉H₃₀O₂)