

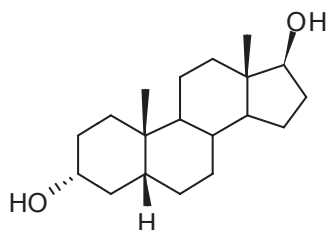


CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: D636.2015.01

Compound Name: **5 β -Androstane-3 α , 17 β -diol**
Collection Number: D636
Chemical Formula: C₁₉H₃₂O₂
CAS Number: 1851-23-6
Structure:

Description: White crystals
Batch Number: 98-001017
Molecular Weight: 292.5
Release Date: 17th December 2002



Synonyms: 3 α , 17 β -Dihydroxy-5 β -androstane
Etiocholan-3 α , 17 β -diol

Purity (mass fraction): 99.7 \pm 1.7% (95% coverage interval)

Purity estimate obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR. Supporting evidence is provided by elemental microanalysis.

GC-FID: Instrument: Agilent 6890N
Column: HP-1, 30 m \times 0.32 mm I.D. \times 0.25 μ m
Program: 215 $^{\circ}$ C (20 min), 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (5 min)
Injector: 250 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
Carrier: Helium Split ratio 20/1

Relative peak area response of 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)
Re-analysis: Mean = 99.6%, s = 0.1% (10 sub samples in duplicate, February 2010)

GC-FID: Instrument: Varian CP-3800
Column: HP-5, 30 m \times 0.32 mm I.D. \times 0.25 μ m
Program: 215 $^{\circ}$ C (20 min), 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (5 min)
Injector: 250 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
Carrier: Helium Split ratio 20/1

Relative peak area response of main component:

Initial analysis: Mean = 99.8%, s = 0.1% (10 sub samples in duplicate, February 2010)

GC-FID: Instrument: Varian CP-3800
Column: VF-1, 30 m \times 0.32 mm I.D. \times 0.25 μ m
Program: 215 $^{\circ}$ C (20 min), 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (5 min)
Injector: 250 $^{\circ}$ C Detector Temp: 320 $^{\circ}$ C
Carrier: Helium Split ratio 20/1

Relative peak area response of main component:

Initial analysis: Mean = 99.5%, s = 0.1% (10 sub samples in duplicate, February 2010)
Initial analysis: Mean = 99.9%, s = 0.04% (10 sub samples in duplicate, February 2010)

Accredited for compliance with ISO Guide 34.

105 Delhi Road North Ryde NSW 2113, PO Box 138 North Ryde NSW 1670 Tel: +61 2 9449 0111 www.measurement.gov.au ABN: 74 599 608 295

GC-FID: Instrument: Varian CP-3800
 Column: HP-1, 30 m × 0.32 mm I.D. × 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 peak area response of main component:
 Initial analysis: Mean = 99.8%, s = 0.1% (10 sub samples in duplicate, February 2010)

GC-FID: Instrument: Varian CP-3800
 (*bis*-TMS Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
 derivative) Program: 230 °C (0.2 min), 5 °C/min to 265 °C, 30 °C/min to 300 °C (3 min)
 Injector: 250 °C Detector Temp: 320 °C
 Carrier: Helium Split ratio 20/1
 Relative peak area response of main component:
 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: HP 5890
 (*bis*-TMS Column: ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm
 derivative) 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 peak area response of main component:
 Initial analysis: Mean = 99.7%, s = 0.03% (10 sub samples in duplicate, April 2010)

Thermogravimetric analysis: Volatiles content not determined.
 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 material is reported along with the major peaks in the mass spectrum. The latter are reported in m/z (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 pellet
Peaks: 3343, 2924, 2864, 1469, 1452, 1367, 1276, 1172, 1056, 1036 cm^{-1}

^1H NMR: Instrument: Bruker DMX-500
Field strength: 500 MHz Solvent: $\text{CDCl}_3/\text{DMSO-d}_6$
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: $\text{CD}_3\text{OD}/\text{DMSO-d}_6$
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)
Calc: C = 78.0%; H = 11.0% (Calculated for $\text{C}_{19}\text{H}_{32}\text{O}_2$)

Expiration of certification

The property values are valid till 21st April 2020, 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.

The long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of five years from the date of certification.

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 annual stability trials.

Homogeneity assessment

The homogeneity of the material was assessed using purity assay by GC-FID on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be 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.

Metrological Traceability

The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis.

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.

Intended Use

For *in vitro* laboratory analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI'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 certificate.

Authorised by:



Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 29 April, 2015.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 29th April 2015.