

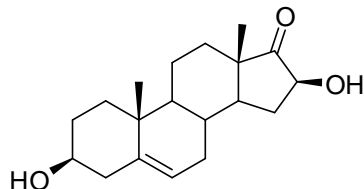


REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D844.2014.01

Compound Name: **16 β -HydroxyDHEA**
Collection Number: D844
Chemical Formula: C₁₉H₂₈O₃
CAS Registry Number: 1159-68-8

Description: White powder
Batch Number: 03-S-14
Molecular Weight: 304.4
Release Date: 6th August 2004 Structure:



Synonyms: 16 β -Hydroxydehydroepiandrosterone

Purity (mass fraction): 99.6 \pm 1.5% (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 HPLC with UV detection, Karl Fischer analysis, thermogravimetric analysis and ¹H NMR. Supporting evidence is provided by elemental microanalysis.

HPLC: Instrument: Waters Model 1525 pump, 717 plus autosampler
Column: Alltech Alltima C-18, 5 μ m (4.6 mm x 150 mm)
Mobile Phase: Acetonitrile/MilliQ Water 35:65
Flow rate: 1.0 mL/min
Detector: PDA, UV at 200 nm
Relative peak area response of main component:
Initial analysis: Mean = 99.8%, s = 0.02% (7 sub samples in duplicate, February 2004)
Re-analysis: Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, August 2008)

HPLC: Instrument: Waters Model 1525 pump, 717 plus autosampler
Column: Alltech Alltima C-18, 5 μ m (4.6 mm x 150 mm)
Mobile Phase: Acetonitrile /MilliQ Water 30:70
Flow rate: 1.0 mL/min
Detector: PDA, UV at 200 nm
Relative peak area response of main component:
Initial analysis: Mean = 99.9%, s = 0.003% (5 sub samples in duplicate, September 2011)
Re-analysis: Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, August 2014)

Thermogravimetric analysis: Volatile content 0.1% and non volatile residue < 0.2 % mass fraction (January 2004 and April 2005)

Karl Fischer analysis: Moisture content < 0.1% mass fraction (August 2008)
Moisture content < 0.1% mass fraction (September 2011)
Moisture content < 0.1% mass fraction (July 2014)

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

Spectroscopic and other characterisation data

GC-MS:	<p>Instrument: Agilent 6890/5973 Column: Ultra 1, 17m x 0.2mm I.D.x 0.11µm Program: 189 °C (0.2 min) 3 °C /min to 240 °C, 10 °C /min to 265, 30 °C/min to 310 °C Injector: 250 °C Transfer line temp: 300 °C Carrier: Helium, 1.0 mL/min Split ratio: 14/1</p> <p>The retention time of the <i>bis</i>-TMS derivative 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.</p> <p>13.7 min: 448 (M⁺, 5), 433 (8), 304 (61), 214 (100), 199 (55), 175 (36), 129 (66), 73 (55) m/z</p>
TLC:	<p>Conditions: Kieselgel 60F₂₅₄. Chloroform/ethyl acetate (4:1) Single spot observed, R_f = 0.20 Visualization with vanillin, H₂SO₄ spray</p>
IR:	<p>Instrument: BioRad FTS3000MX FT-IR Range: 4000-400 cm⁻¹, KBr powder Peaks: 3402, 3317, 2930, 1732, 1459, 1433, 1403, 1373, 1300, 1048, 963, 912 cm⁻¹</p>
¹ H NMR:	<p>Instrument: Bruker DMX-500 Field strength: 500 MHz Solvent: DMSO d₆ (2.5 ppm) Key spectral data: δ 0.82 (3H, s), 0.95 (3H, s), 3.24 (1H, m), 3.76 (1H, m), 4.60 (1H, d), 5.27 (1H, bd), 5.61 (1H, d) ppm</p>
¹³ C NMR:	<p>Instrument: Bruker Gyro-300 Field strength: 75.5 MHz Solvent: CDCl₃ (77.0 ppm) Spectral data: δ 14.7, 19.4, 20.1, 30.6, 30.6, 31.0, 31.6, 31.7, 36.8, 37.1, 42.2, 46.1, 46.7, 50.5, 71.6, 75.4, 120.8, 141.1, 220.1 ppm</p>
Melting point:	197-204 °C
Microanalysis:	<p>Found: C = 75.0%; H = 9.3% Calc: C = 75.0%; H = 9.3% (Calculated for C₁₉H₂₈O₃)</p>

Expiration of certification

The property values are valid till 27th August 2019, i.e. five years from the date of recertification 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 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 HPLC-UV on seven 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.

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:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 3 September, 2014.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 2nd September 2014.