Australian Government

National Measurement Institute

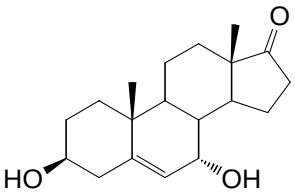
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D875.2012.02

Compound Name: **7α-HydroxyDHEA**
Collection Number: D875
Chemical Formula: $C_{19}H_{28}O_3$
CAS Registry Number: 53-00-9

Description: White powder
Batch Number: 03-S-11
Molecular Weight: 304.4
Batch Production Completed: May 2004

Structure:



Synonyms: 7α-Hydroxydehydroepiandrosterone

Purity (mass fraction): 96.5 ± 1.6% (95% coverage interval)

The purity value was 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, and Karl Fischer analysis. Supporting evidence is provided by ^{1}H NMR and elemental microanalysis.

GC-FID

Instrument:	Agilent 5890 or 6890N	
Column:	Zebron ZB-1 or HP-1, 29.88 m x 0.32 mm I.D. x 0.25 µm	
Program	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2 min)	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area response of main component:

Initial analysis:	Mean = 98.5%, s = 0.05% (7 sub samples in duplicate, May 2004)
Re-analysis:	Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, October, 2007)
Re-analysis:	Mean = 98.6%, s = 0.08% (5 sub samples in duplicate, March 2009)
Re-analysis:	Mean = 99.0%, s = 0.05% (5 sub samples in duplicate, February, 2012)

Thermogravimetric analysis: Volatile content 2.1% and non-volatile content < 0.2% mass fraction. (June 2004 and October 2005)
Volatile content 1.9% and non-volatile content < 0.2% mass fraction (October 2007)

Karl Fischer analysis: Moisture content 2.7% mass fraction (February 2007)
Moisture content 3.2% mass fraction (March 2009)
Moisture content 2.6% mass fraction (February 2012)

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973	
Column:	ZB-5, 30 m x 0.25 mm I.D. x 0.30 µm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium 1.0 mL/min	Split ratio: 20/1

Tris-TMS Derivative:

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 °C, 30 °C/min to 310 °C (2 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 14/1

The retention times of the parent and the *tris*-TMS derivative are reported along with the major peaks observed in the mass spectrum. The latter are reported as mass to charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.4 min): 304 (M^+, 4), 286 (100), 271 (10), 145 (11), 143 (11), 119 (11), 105 (12), 91 (16), 79 (11), 55 (9) m/z

Tris-TMS(11.2 min):520 (5), 430 (35), 415 (49), 325 (33), 250 (6), 235 (8), 195 (8), 181 (17), 169 (65), 129 (12), 73 (100) m/z

TLC: Conditions: Kieselgel 60F_{254}. 100% Ethyl acetate, single spot observed R_f = 0.19. Visualization with vanillin, H_2SO_4 spray

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3430, 3303, 2930, 2860, 1724, 1456, 1438, 1376, 1059, 1029, 1012, 953 cm^{-1}

^{1}H NMR:

Instrument:	Gyro 300	
Field strength:	300 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.89 (3H, s), 1.02 (3H, s), 3.58 (1H, m), 3.97 (1H, m), 5.64 (1H, dd, *J* = 1.9, 5.3 Hz) ppm	

^{13}C NMR:

Instrument:	Bruker Advance 300	
Field strength:	75.5 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.3, 18.3, 20.1, 21.9, 31.1, 31.3, 35.8, 37.0, 37.2, 37.5, 41.9, 42.7, 45.0, 47.1, 64.3, 71.2, 123.6, 146.6, 221.0 ppm	

Microanalysis: Found: C = 73.1%, H = 9.8% (April 2009)
Calc: C = 75.0%, H = 9.3% (Calculated for $C_{19}H_{28}O_3$)

Melting point: 175-177 °C (Lit. 180-181 °C)

Expiration of certification

The property values are valid till 20th February 2017, i.e. five years from the date of 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/ampoules 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 GC-FID on 5 randomly selected 1-2 mg 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 ºC 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: 26 July, 2012

Characterisation data and property values specified in this report supersede those in all reports issued prior to 18th July 2012.