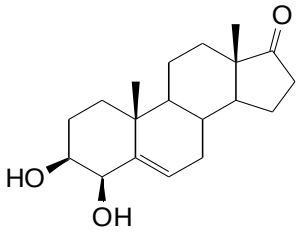
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D834.2016.01 (Ampouled 050505)
This batch of ampoules was prepared from the bulk material on 5th May 2005.

Compound Name: **4β-Hydroxydehydroepiandrosterone**
Collection Number: D834
Chemical Formula: $C_{19}H_{28}O_3$
CAS Number: 63518-24-1
Description: White crystals
Batch Number: 03-S-13
Molecular Weight: 304.4
Release Date: June 2003

Structure:



Synonyms: 3β, 4β-Dihydroxyandrost-5-ene-17-one,
4β-hydroxy-DHEA

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 D834. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 996 ± 6 μg of anhydrous 4β-hydroxydehydroepiandrosterone. The uncertainty is stated at the 95% coverage interval.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	A = MilliQ Water; B = Acetonitrile 0-8 min 40% B; 8-9 min 40-60% B; 9-15 min 60% B; 15-16 min 60-40% B; 16-22 min 40% B.
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at 201 nm [2005, 2006, 2007]/Waters 2998 PDA operating at 201 nm [2011, 2016]
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.6%, s = 0.005% (3 ampoules in duplicate, May 2005)
	Re-analysis:	Mean = 99.5%, s = 0.02% (3 ampoules in duplicate, May 2006)
	Re-analysis:	Mean = 99.5%, s = 0.02% (5 ampoules in duplicate, May 2007)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, December 2011)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, November 2016)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

Purity estimate was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis and Karl Fischer analysis. Supporting evidence is provided by elemental microanalysis and ^{1}H NMR spectroscopy.

HPLC:	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/Water (40:60 v/v)
	Flow Rate:	1.0 mL/min
	Detector:	UV at 201 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.6%, s = 0.02% (7 sub samples in duplicate, July 2003)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 sub samples in duplicate, May 2005)
GC-MS:	Instrument:	HP 5890/5971A
	Column:	BPX-5, 30 m × 0.22 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (5 min)
	Injector:	230 °C Transfer line temp: 320 °C
	Carrier:	Helium Split ratio: 20/1

The retention time of the *tris*-TMS derivative is reported along 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.
13.8 min: 520 (M^+, 1), 195 (6), 169 (16), 147 (17), 129 (13), 73 (100) *m/z*

TLC:	Conditions:	Kieselgel 60F_{254}. Hexanes/Ethyl acetate (1:1) Single spot observed, R_f = 0.25
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3335, 2943, 1744, 1662, 1449, 1080, 1055, 967cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: CD_3OD (3.31 ppm)
	Key spectral data:	δ 0.94 (3H, s), 1.27 (3H, s), 2.50 (1H, dd), 3.50 (1H, ddd), 4.12 (1H, d), 5.71 (1H, dd) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz Solvent: $CDCl_3$ (77.16 ppm)
	Spectral data:	δ 13.5, 19.8, 20.9, 21.8, 25.2, 30.9, 31.3, 31.4, 35.8, 36.1, 36.9, 47.5, 50.3, 51.9, 72.3, 77.1, 127.7, 142.9, 221.0 ppm
Melting point:		197-200 °C
Microanalysis:		Found: C = 74.9%; H = 9.5% (May 2003) Calc: C = 74.9%; H = 9.3% (Calculated for $C_{19}H_{28}O_3$)
Thermogravimetric analysis:		Volatiles content and non-volatile residue < 0.3% total (October 2003 & May 2005)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (July 2003)

Expiration of certification
The property values are valid till 17th November 2021, 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.

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 with UV detection on five 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.

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: 23 November, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 23rd November, 2016.