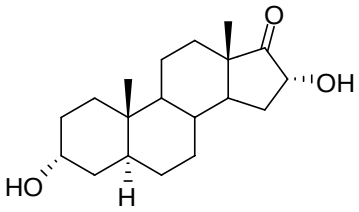
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D843.2014.01 (Ampouled 050707)
This batch of ampoules was prepared from the bulk material on 7th July 2005.

Compound Name: **16α-Hydroxyandrosterone**
Collection Number: D843
Chemical Formula: $C_{19}H_{30}O_3$
CAS Registry Number: 14167-49-8
Structure:



Description: White crystals
Batch Number: 03-S-09
Molecular Weight: 306.4
Release Date: 4th February2004

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 D843. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (methanol). This will transfer 984 ± 66 µg of anhydrous 16α-hydroxyandrosterone. The uncertainty is stated at the 95% coverage interval.

HPLC:	Instrument:	Waters 1525 binary pump, W717 autosampler
	Column:	Alltima C-18 5 µm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/Water (42:58)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 or 2998 operating at 205 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (3 ampoules in duplicate, July 2005)
	Re-analysis:	Mean = 99.7%, s = 0.04% (5 ampoules in duplicate, August 2006)
	Re-analysis:	Mean = 99.8%, s = 0.03% (5 ampoules in duplicate, August 2009)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, July 2014)
	Detector:	Waters ELSD 2420
	Initial analysis:	Mean = 99.98%, s = 0.01% (5 ampoules in duplicate, August 2009)

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

Purity estimate obtained by subtraction from 100% of total impurities by HPLC with UV detection at 205 nm, thermogravimetric analysis and ^{1}H NMR. Supporting evidence was provided by elemental microanalysis

HPLC:	Instrument:	Waters 1525 binary pump, W717 autosampler
	Column:	Alltima C-18 5 µm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/ Water (42:58)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at 205 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.6%, s = 0.03% (7 sub samples in duplicate, October 2003)
	Current re-analysis:	Mean = 99.7%, s = 0.04% (5 sub samples in duplicate, August 2005)
GC-MS:	*Bis*-TMS derivative:	
	Instrument:	HP6890/5973
	Column:	Ultra 1, 17 m x 0.20 mm ID x 0.11 µm
	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 1mL/min Split ratio: 14/1
	The retention time of the *bis*-TMS derivative is reported with the major peaks in the mass spectrum. The latter are reported in mass / charge ratios and (in brackets) as a percentage relative to the intensity of the base peak.	
	11.5 min:	450 (M^+, 2), 435 (4), 306 (51), 216 (100), 201 (54), 117 (74), 106 (58), 73 (60) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/Ethyl acetate (1:1) Single spot observed, R_f = 0.17
IR:	Instrument:	FT-IR, Biorad FTS3000MX
	Range:	4000-400 cm^{-1}, KBr
	Peaks:	3517, 3348, 1739, 1447, 1247, 1150, 1014, 999, 929, 907, 617 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: DMSO-d_6
	Key spectral data:	δ 0.74 (3H, s), 0.82 (3H, s), 0.91 (1H, m), 1.86 (1H, m), 3.80 (1H, br s), 4.18 (2H, m), 5.30 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz Solvent: DMSO-d_6
	Spectral data:	δ 11.5, 14.4, 19.7, 28.4, 29.0, 30.8, 31.9, 32.2, 34.8, 36.1, 36.1, 38.9, 47.4, 48.4, 54.4, 64.4, 70.5, 219.4 ppm
Melting point:		202-205 °C
Microanalysis:		Found: C = 74.4%, H = 9.9% (August 2003) Calc: C = 74.5%, H = 9.9% (Calculated for $C_{19}H_{30}O_3$)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction. (October 2003 & August 2006)

Expiration of certification
The property values are valid till 18th July 2019, 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-UV on five randomly selected 1 mg ampoules 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: 24 July, 2014.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 24th July 2014.