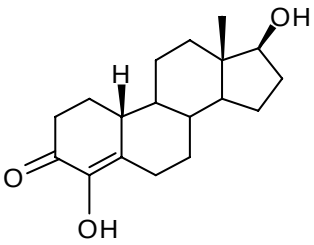
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D853b.2011.02 (Ampouled 091001)
This batch of ampoules was prepared from the bulk material on 1st October 2009.

Compound Name: **4-Hydroxynandrolone**
Collection Number: D853b
Chemical Formula: $C_{18}H_{26}O_3$
CAS Registry Number: 4721-69-1
Structure:



Description: White powder
Batch Number: 07-S-04
Molecular Weight: 290.4
Batch Production Completed: May 2009

Synonyms: Oxabolone
4-Hydroxynortestosterone
4,17β-Dihydroxyestr-4-en-3-on

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 D853b. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile). This will transfer 0.989 ± 0.012 mg of anhydrous 4-hydroxynandrolone. The uncertainty is stated at the 95% coverage interval.

Note: Estradiol was confirmed as the major impurity in this material. Due to the large difference in the extinction coefficient of estradiol compared with 4-hydroxynandrolone in the HPLC analysis, the final purity assessment used the estradiol content determined by ^{1}H NMR.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5µm (4.6 mm × 150 mm)
	Column oven:	30 °C
	Mobile Phase:	A: MilliQ water, B: Acetonitrile 70% A for 32 minutes, decrease A to 20% in 2 minutes, 20% A for 4 minutes, increase A to 70% in 2 minutes, 70% A for 5 minutes
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at Max plot
	Retention time:	18.7 min
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.6%, s = 0.02% (7 ampoules in duplicate, October 2009)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5µm (4.6 mm × 150 mm)
	Column oven:	30 °C
	Mobile Phase:	A: Acetonitrile, B: MilliQ water 30% A for 32 minutes, increase A to 80% in 2 minutes, 80% A for 4 minutes, decrease A to 30% in 2 minutes, 30% A for 5 minutes
	Flow Rate:	1.0 mL/min
	Detector:	Shimadzu PDA SPD-M20A operating at Max plot
	Retention time:	18.5 min [2010], 16.5 min [2012]
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (5 ampoules in duplicate, December 2010)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, January 2012)

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

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, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/MilliQ water (30:70)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at Max plot
	Retention time:	19.3 min
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.5%, s = 0.07% (7 sub samples in duplicate, May 2009)
Thermogravimetric analysis:		Initial volatile content 0.28% and non volatile residue < 0.2 % mass fraction (May 2009)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (May 2009)

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973	
Column:	VF-1ms, 14.9 m x 0.25 mm I.D. x 0.25 µm	
Program:	180 °C (1 min), 10 °C/min to 300 °C (1 min)	
Injector:	180 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 30/1

The retention time of the parent compound is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (7.8 min): 290 (M^+, 100), 272 (28), 243 (8), 218 (9), 176 (11), 161 (13), 147 (34), 126 (48), 91 (38), 79 (38), 67 (29), 55 (32), 41 (29) m/z

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1)
Single spot observed, R_f = 0.38. Visualisation with UV at 254 nm

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3542, 3417, 2947, 2913, 1664, 1650, 1653, 1622, 1389, 1351, 1158, 1063, 1052, 876, 618 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III 500	
Field strength:	500 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.79 (3H, s), 0.81 (1H, ddd, *J* = 3.8, 10.5, 10.5 Hz), 0.94-1.023 (2H, m), 1.11 (1H, m), 1.19-1.14 (3H, m), 1.41-1.52- (2H, m), 1.63 (1H, m), 1.79-1.94 (4H, m), 2.03-2.18 (2H, m), 2.24 (1H, m), 2.34 (1H, m), 2.56 (1H, ddd, *J* = 4.1, 4.1, 17.1 Hz), 3.12 (1H, m), 3.66 (1H, t, *J* = 8.6), 6.10 (1H s) ppm. Ethyl acetate at 0.48% mass fraction was observed in the ^{1}H NMR Estradiol at 0.53% mass fraction was observed in the ^{1}H NMR	

^{13}C NMR:

Instrument:	Bruker Gyro-300	
Field strength:	75 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 11.0, 23.2, 25.88, 25.92, 26.1, 29.5, 30.5, 34.5, 36.4, 40.0, 41.5, 42.9, 49.6, 49.8, 81.8, 135.3, 142.0, 194.1 ppm	

Melting point: 180-183 °C

Microanalysis: Found: C = 74.3 %; H = 8.8% (May, 2009)
Calc: C = 74.5 %; H = 9.0 % (Calculated for $C_{18}H_{26}O_3$)

Expiration of certification
The property values are valid till 4th January 2015, i.e. three 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 been given a shelf life of three years from the date of re-certification. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years.

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 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: 14 August, 2012.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 2nd August 2012.