Australian Government
National Measurement Institute

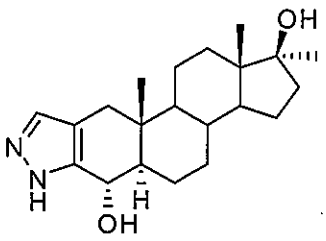
NATA TECHNICAL COMPETENCE

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D642.2016.01 (Ampouled 060525)
This batch of ampoules was prepared from the bulk material on 25th May 2006.

Compound Name: **4α-Hydroxystanozolol**
Collection Number: D642
Chemical Formula: $C_{21}H_{32}N_2O_2$
CAS Number: N/A
Structure:

Description: Colorless solid
Batch Number: 00-S-08
Formula Weight: 344.5
Release date: 2nd July 2002



Synonym: 4α, 17β-Dihydroxy-17α-methyl-5α-androstano [3,2c]pyrazole hydrate

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 D642. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 948 ± 24 µg of anhydrous 4α-hydroxystanozolol.The uncertainty is stated at the 95% coverage interval.

HPLC:

Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
Column:	Alltech Alltima C-18, 5 µm (4.6 mm × 150 mm)
Mobile Phase:	Methanol/water (65:35)
Flow Rate:	1.0 mL/min
Detector:	Waters ELSD 2424

Relative peak area response of main component:

Initial analysis:	Mean = 99.6%, s = 0.01% (7 ampoules in duplicate, November 2006)
Re-analysis:	Mean = 99.7%, s = 0.004% (5 ampoules in duplicate, November 2007)
Re-analysis:	Mean = 99.7%, s = 0.00% (5 ampoules in duplicate, February 2011)
Re-analysis:	Mean = 99.6%, s = 0.03% (5 ampoules in duplicate, December 2013)
Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, November 2016)

Detector: Waters 2998 PDA operating at 226 nm

Relative peak area response of main component:

Initial analysis:	Mean = 97.7%, s = 0.04% (7 ampoules in duplicate, November 2006)
Re-analysis:	Mean = 97.8%, s = 0.04% (5 ampoules in duplicate, November 2007)
Re-analysis:	Mean = 97.7%, s = 0.01% (5 ampoules in duplicate, February 2011)
Re-analysis:	Mean = 97.7%, s = 0.02% (5 ampoules in duplicate, December 2013)
Re-analysis:	Mean = 97.7%, s = 0.05% (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 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-ELSD, thermogravimetric analysis and Karl-Fischer analysis. Supporting evidence is provided by elemental microanalysis and ^{1}H NMR.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltech Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Methanol/water (65:35)
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (3 sub samples in duplicate, May 2003)
	Re-analysis:	Mean = 99.9%, s = 0.02% (2 sub samples in duplicate, June 2004)
	Detector:	UV at 226 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean > 98% (1 sub sample, March 2000)
	Re analysis:	Mean = 98.0%, s = 0.04% (3 sub samples in duplicate, May 2003)
	Re analysis:	Mean = 97.9%, s = 0.3% (2 sub samples in duplicate, June 2004)
Thermogravimetric analysis:		Initial volatile content 2.0% and non volatile residue < 0.2% mass fraction (March 2000) Volatile content 2.3% (June 2006)
Karl Fischer analysis:		Moisture content 3.7% mass fraction (November 2006)

Spectroscopic and other characterisation data

GC-MS:	*Tris*-TMS derivative:		
	Instrument:	HP6890/5973	
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.22 μm	
	Program:	180 °C (0.5min) 12°C/min to 310 °C (3 min)	
	Injector:	260 °C	Transfer line temp: 300 °C
	Carrier:	Helium, 1.0 mL/min	Split ratio: 30/1
	Relative peak area response of main component = 99.5% (mean of 5 samples)		
	The retention time of the *tris*-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 intensity of the base peak.		
	Tris-TMS (9.07 min): 560 (M^+, 40), 545 (39), 471 (14), 254 (56), 143 (100), 73 (85) m/z		
ESI-MS:	Instrument:	Finnigan MAT TSQ 700 with electrospray interface	
	Operation:	Negative ion mode and positive ion mode, direct infusion at 5μL/min	
	Ionisation:	ESI spray voltage 2.5 kV for -ve ion mode, 4.5kV for +ve ion mode	
	Peaks:	403.4 (MCH_3COO^-, 100), 343.3 (M^-), 325.0 ($M^- - H_2O$) m/z (-ve ion mode) 345.3 (MH^+, 100), 327.3 ($MH^+ - H_2O$), 309.3 ($MH^+ - 2H_2O$) m/z (+ve ion mode)	
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (90:10)	
		Single spot observed	R_f = 0.32 (3 samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS-3000MX	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	3290 (br), 1447, 1372, 1293, 1156, 1067, 947 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker ARX-500	
	Field strength:	500 MHz	Solvent: d_6-DMSO (2.50 ppm)
	Key spectral data:	δ 0.67 (3H, s), 0.74 (3H, s), 1.07 (3H, s), 4.04 (1H, s) 7.19 (1H, br s) ppm	
^{13}C NMR:	Instrument:	Bruker ARX-500	
	Field strength:	126 MHz	Solvent: d_6-DMSO (39.52 ppm)
	Spectral data:	δ 12.4, 14.1, 20.1, 23.2, 24.3, 26.1, 31.0, 31.4, 34.4, 35.9, 38.4, 45.0, 50.1, 51.0, 53.4, 65.7, 79.7, 113.6, 136.3, 139.7 ppm	
		One signal obscured by the solvent peak.	
Melting point:		172 °C	
Microanalysis:		Found: C = 70.1%; H = 9.2%; N = 7.6% (April 2000)	
		Calc: C = 73.2%; H = 9.4%; N = 8.1% (Calculated for $C_{21}H_{32}N_2O_2$)	

Expiration of certification
The property values are valid till 9th 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 ELS detection on seven 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 °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: 25 November, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 25th November 2016.

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