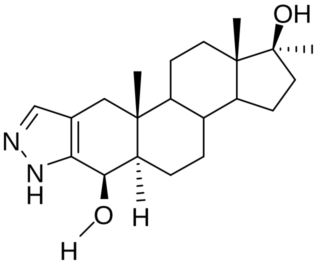
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D641: 4β-Hydroxystanozolol



Report ID: D641.2016.02 (Ampouled 070529)

Chemical Formula: $C_{21}H_{32}N_2O_2$

Molecular Weight: 344.5 g/mol

Certified value

Batch No.	CAS No.	Mass per ampoule
00-S-07	**125636-92-2**	**464 ± 19 µg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (1S,3aS,3bR,5aR,6R,10aR,10bS,12aS)-1,10a,12a-Trimethyl-1,2,3,3a,3b,4,5,5a,6,7,10,10a,10b,11,12,12a-hexadecahydrocyclopenta[5,6]naphtho[1,2-f]indazole-1,6-diol.

Expiration of certification: The property values are valid till 23 May 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D641. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 464 ± 19 µg of 4β-Hydroxystanozolol. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV and ELSD 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.

Safety: Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
19 March 2020

This report supersedes any issued prior to 19 March 2020.

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

HPLC:	Instrument:	Waters alliance 2650
	Column:	Alltech Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot/254 nm Waters ELSD 2420
	Retention time:	UV = 17.2 min, ELSD = 17.2 min
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9 % (3 sub samples in duplicate, June 2000)
	Re-analysis:	Mean = 99.5 %, s = 0.01 % (UV, 7 ampoules in duplicate, June 2007) Mean = 99.9 %, s = 0.04 % (ELSD, 7 ampoules in duplicate, June 2007)
	Re-analysis:	Mean = 99.5 %, s = 0.01 % (UV, 5 ampoules in duplicate, June 2008) Mean = 99.9 %, s = 0.02 % (ELSD, 5 ampoules in duplicate, June 2008)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Grace Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	50 °C
	Mobile Phase:	Methanol/water (60:40)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot/254 nm Waters ELSD 2420
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.6 %, s = 0.01 % (UV, 5 ampoules in duplicate, July 2011) Mean = 99.9 %, s = 0.03 % (ELSD, 5 ampoules in duplicate, July 2011)
	Re-analysis:	Mean = 99.7 %, s = 0.01 % (UV, 5 ampoules in duplicate, May 2016)

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

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV/ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\ \% - I_{\text{ORG}}) \times (100\ \% - I_{\text{VOL}} - I_{\text{NVR}}) \qquad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters alliance 2650
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (70:30 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot/254 nm Waters ELSD 2420
	Retention time:	UV = 10.4 min, ELSD = 10.5 min
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9 % (3 sub samples in duplicate, June 2000)
	Re-analysis:	Mean = 99.6 %, s = 0.02 % (UV, 7 sub samples in duplicate, June 2007) Mean = 99.9 %, s = 0.04 % (ELSD, 7 sub samples in duplicate, June 2007)

Karl Fischer analysis:	Moisture content 0.6 % mass fraction (2 sub samples, June 2007)
Thermogravimetric analysis:	Non-volatile residue < 0.2 % mass fraction (November 2005 & July 2007). Volatile content not determined by TGA

Spectroscopic and other characterisation data

GC-MS:	*Tris*-TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.22 µm
	Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (2 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	The retention time of the *tris*-TMS derivative 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.	
	Tris-TMS (9.7 min):	560 (M^+, 38), 545 (23), 470 (7), 254 (49), 143 (98), 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (90:10) Single spot observed, R_f = 0.32 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS-3000MX
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3280, 3164, 1579, 1444, 1372, 1294, 1156, 1067, 947 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.5 ppm)
	Key spectral data:	δ 0.74 (3H, s), 0.85 (3H, s), 1.07 (3H, s), 4.04 (1H, s) ppm
		Absorbances due to pyridine observed at δ 7.0, 7.35 and 8.5 ppm^{13}C NMR:
	Instrument:	Bruker ARX-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 14.1, 14.2, 20.0, 23.2, 24.7, 26.0, 26.1, 31.4, 31.5, 35.1, 36.2, 36.2, 38.3, 38.4, 45.0, 48.1, 50.0, 54.0, 79.6, 79.8, 113.3 ppm
		Absorbances due to pyridine observed at δ 123.9, 136.1, 149.6 ppm
Melting point:		277-278 °C
Microanalysis:	Found:	C = 73.3 %; H = 9.3 %; N = 9.1 % (April 2000)
	Found:	C = 73.6 %; H = 9.4 %; N = 9.1 % (July 2006)
	Calculated:	C = 73.5 %; H = 9.1 %; N = 9.1 % (for $C_{21}H_{32}N_2O_2$.½C_5H_5N)