Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

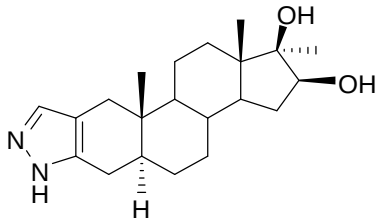
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D621: 16β-Hydroxystanozolol

Report ID: D621.2018.02

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

Molecular Weight: 344.5 g/mol



Property value

Batch No.	CAS No.	Purity estimate
99-S-30	125590-76-3	84.6 %

IUPAC name: (1R,2S,3aS,3bR,5aS,10aS,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-1,2-diol

Expiration of certification: The property values are valid till 22 August 2023, 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White crystals prepared by sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This reference material should be used for qualitative analysis only.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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.

Stability: This material has demonstrated stability over a minimum period of five years. The long-term stability of the compound in solution has not been examined.

Safety: Treat as a 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.
27 March 2020

This report supersedes any issued prior to 27 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:

The purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The three-proton triplet at 3.9 ppm was measured against a certified internal standard of triazine. Supporting evidence is provided by elemental microanalysis, HPLC with UV detection, Karl Fischer analysis and thermogravimetric analysis.

QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	Pyridine-d_5
	Internal standard:	Triazine
	Purity estimate:	84.6% (mass fraction %, mean of five samples, s = 0.25%, July 2009)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus
	Column:	Alltima C-18, 5 μm (4.6 mm x 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 224 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 93.1%, s = 0.2% (5 sub samples in duplicate, September 2013)
	Re-analysis:	Mean = 93.3%, s = 0.3% (5 sub samples in duplicate, August 2018)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus
	Column:	Alltima C-18, 5 μm (2.0 mm x 150 mm)
	Mobile Phase:	Methanol/MilliQ water (70:30 v/v)
	Flow rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	> 99% (7 sub samples, October 1999)
	Re-analysis:	Mean = 98.4%, s = 0.1% (8 sub samples in duplicate, May 2006)
	Detector:	PDA operating at 225 nm
	Relative peak area of the main component:	
	Initial analysis:	> 99% (7 sub samples, October 1999)
	Re-analysis:	Mean = 94.5%, s = 0.3% (8 sub samples in duplicate, May 2006)
Karl Fischer analysis:		Moisture content 0.5% mass fraction (April 2007 and April 2009) Moisture content 0.6% mass fraction (September 2013) Moisture content 0.7% mass fraction (August 2018)
Thermogravimetric analysis:		Volatiles content and non-volatile residue < 0.3% mass fraction (May 2006)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 µm
	Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	The retention time of the *bis*-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.	
	Parent (7.8 min):	560 (M^+, 21), 488 (1), 470 (5), 381 (7), 218 (44), 147 (20), 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate Single spot observed, R_f = 0.4
		Kieselgel 60F254. Methanol/chloroform (1:4) Single spot observed, Rf = 0.63
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3252, 1594, 1520, 1447, 1382, 1178, 1045, 967 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX-500
	Field strength:	500 MHz
	Solvent:	d_5-Pyridine
	Key Spectral data:	δ 0.78 (3H, s), 1.15 (3H, s), 1.32 (3H, s), 3.92 (1H, dd), 7.65 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker ARX-500
	Field strength:	126 MHz
	Solvent:	d_5-Pyridine
	Spectral data:	δ 11.7, 14.4, 21.0, 24.7, 27.2, 29.5, 31.9, 32.0, 33.1, 35.3, 35.9, 36.4, 36.9, 38.3, 42.9, 45.4, 47.6, 54.2, 77.4, 78.9, 114.4 ppm
Melting point:		295-300 °C
Microanalysis:	Found:	C = 72.2%, H = 9.7%, N = 7.6% (October 1999)
	Found:	C = 71.5%, H = 9.6%, N = 7.9% (May 2007)
	Calculated:	C = 73.2%, H = 9.4%, N = 7.4% (Calculated for $C_{21}H_{32}N_2O_2$)