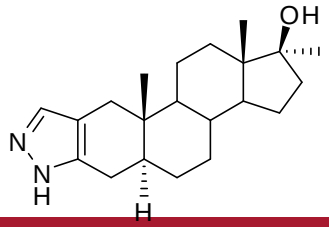
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D646: Stanozolol

Report ID: D646.2021.01 (Bottled 160202)

Chemical Formula: $C_{21}H_{32}N_2O$

Molecular Weight: 328.5 g/mol



Property value

Batch No.	CAS No.	Purity estimate
00-S-09	10418-03-8	98%

IUPAC name: (1S,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]indazol-1-ol

Expiration of certification: The property values are valid till 7 June 2024, 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 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 powder 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 three years. 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 detection on five randomly selected 1-2 mg sub samples 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 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.
22 June 2021

This report supersedes any issued prior to 22 June 2021

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 14214. 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 identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. Impurities of related structure were assessed by HPLC with UV detection. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

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

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

HPLC:	Instrument:	
	Column:	Alltech Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (70:30)
	Flow Rate:	0.8 mL/min
	Detector:	ELSD and PDA at 225 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.1% (3 sub samples, February 2000)
	Re-analysis (ELSD):	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, September 2006)
	Re-analysis (UV):	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, September 2006)
HPLC:	Instrument:	Waters Alliance 2695
	Column:	Grace Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (70:30)
	Flow Rate:	0.8 mL/min
	Detector:	PDA at 225 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, October 2011)
	Re-analysis:	Mean = 99.2%, s= 0.05% (5 sub samples in duplicate, July 2016)
	Re-analysis:	Mean = 99.4%, s= 0.02% (5 sub samples in duplicate, June 2021)
Karl Fischer analysis:		Moisture content 0.9% mass fraction (September 2011 and August 2016) Moisture content 1.3% mass fraction (May 2021)
Thermogravimetric analysis:		Volatile content 0.34% mass fraction. Non-volatile residue < 0.2% mass fraction (May 2000, October 2006 and September 2011)

Spectroscopic and other characterisation data

GC-MS: *Bis*-TMS derivative:

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.

Bis-TMS (7.4 min): 472 (M^+, 42), 457 (13), 342 (17), 168 (20), 143 (100), 73 (99) *m/z*

The bis-TMS derivative co-elutes with a silylated comparison sample of stanozolol and the two materials give matching mass spectra

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3300, 1597, 1449, 1371, 1345, 1084, 966, 935 cm^{-1}

^{1}H NMR:

Instrument:	Bruker ARX-500
Field strength:	500 MHz
Solvent:	Acetone-d_6 (2.05 ppm)
Key Spectral data:	δ 0.77 (3H, s), 0.88 (3H, s), 1.19 (3H, s), 7.28 (1H, s) ppm

^{13}C NMR:

Instrument:	Bruker ARX-500
Field strength:	126 MHz
Solvent:	Acetone-d_6 (29.8 ppm)
Key Spectral data:	δ 11.8, 14.5, 21.6, 24.1, 26.5, 27.3, 32.4, 32.6, 35.8, 37.3, 37.6, 39.5, 43.6, 46.3, 51.6, 54.9, 81.1, 132.4 ppm

Melting point: 232-237 °C

Microanalysis:

Found:	C = 76.7%, H = 9.9%, N = 8.5% (April 2000)
Calculated:	C = 76.8%, H = 9.8%, N = 8.5% (Calculated for $C_{21}H_{32}N_2O$)