Australian Government

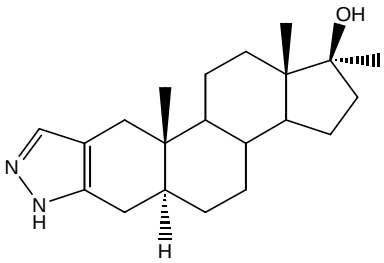
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D646.2011.02

Compound Name: **Stanozolol**
Collection Number: D646
Chemical Formula: $C_{21}H_{32}N_2O$
CAS Number: 10418-03-8
Structure:

Description: White crystals
Batch Number: 00-S-09
Molecular Weight: 328.5
Batch Production Completed: February 2000



Synonyms: Androstanazole, Stanazol, Stromba, Winstrol
17β-Hydroxy-17α-methyl-5α-androstano[3,2-c]pyrazole

Purity (mass fraction): 98.5 ± 2.6% (95% coverage interval)

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

HPLC:
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 U.V. detection at 225 nm
Retention time: 9.9 min
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:
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: U.V. detection at 225 nm
Retention time: 6.9 min
Relative peak area response of main component:
Initial analysis: Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, October 2011)

Thermogravimetric analysis: Volatile content 0.34% mass fraction
Non-volatile residue < 0.2% mass fraction (May 2000, October 2006 and September 2011)

Karl Fischer analysis: Moisture content 0.92% mass fraction (September 2011)

Spectroscopic and other characterisation data

GC-MS: *Bis*-trimethylsilyl derivative:

Instrument:	HP6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm	
Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)	
Injector:	280 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0mL/min	Split ratio 15/1

The retention time of the *bis*-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 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
Key peaks:	3300, 1597, 1449, 1371, 1345, 1084, 966, 935 cm^{-1}

^{1}H NMR:

Instrument:	Bruker ARX-500	
Field strength:	500 MHz	Solvent: d_6-Acetone (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: d_6-Acetone (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	

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

Melting point: 232-237 °C

Expiration of certification
The property values are valid till 7th October 2016, 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.

The long-term stability of the compound in solution has not been examined.

This material has demonstrated stability over a minimum period of 5 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 UV detection on five randomly selected 1-2 mg sub samples 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: 2 August, 2012.

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