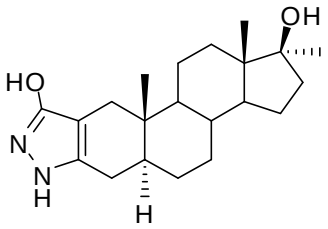
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D577: 3'-Hydroxystanozolol

Report ID: D577.2020.01 (Ampouled 120320)

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

Molecular Weight: 344.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-06	**125709-39-9**	**910 μg**

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]indazole-1,9-diol.

Expiration of certification: The property values are valid till 11 November 2023, 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 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 and is intended for a single use to prepare a standard solution containing D577. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

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

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 approximately 910 μg of anhydrous 3'-hydroxystanozolol.

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 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.
25 November 2020

This report supersedes any issued prior to 25 November 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 Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven:	50 °C
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate, pH 4.2 (37:63)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 248 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.3%, s = 0.3% (7 ampoules in duplicate, March 2012)
	Re-analysis:	Mean = 98.3%, s = 0.03% (4 ampoules in duplicate, February 2013)
	Re-analysis:	Mean = 98.9%, s = 0.07% (5 ampoules in duplicate, February 2014)
	Re-analysis:	Mean = 96.8%, s = 0.3% (6 ampoules in duplicate, February 2017)
	Re-analysis:	Mean = 97.2%, s = 0.2% (5 ampoules in duplicate, February 2018)
	Re-analysis:	Mean = 96.0%, s = 0.3% (5 ampoules in duplicate, November 2020)

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 purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection at 248 nm, thermogravimetric analysis, and Karl Fischer analysis. All organic impurities are assumed to have identical molar extinction coefficients at 248 nm and molecular weights as 3′-hydroxystanozolol. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by qualitative elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven:	50 °C
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate, pH 4.2 (37:63)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 248 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.02% (10 sub samples in duplicate, November 1999)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, April 2005)
	Re-analysis:	Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, March 2012)
Karl Fischer analysis:		Moisture content 6.6% mass fraction (December 2007) Moisture content 6.0% mass fraction (March 2012)
Thermogravimetric analysis:		Volatiles content 2.5 % and non-volatile residue < 0.2% mass fraction (December 2007)

Spectroscopic and other characterisation data

GC-MS:	*Tris*-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m x 0.20 mm I.D. x 0.10 μm
	Program:	170 °C (0.5 min), 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 *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 (16.7 min):	560 (M^+, 36), 545 (47), 254 (56), 143 (100), 73 (99)
ESI-MS:	Instrument:	Finnigan MAT TSQ 700 with electrospray interface
	Operation:	Positive ion mode and negative ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion mode, at 3.0 kV negative ion mode
	EM voltage:	650 V
	Cone voltage:	10 V
	Peak:	403.2, 390.3, 373.3, 343.3 (M-H, 100) *m/z* (negative ion mode) 345.2 (MH^+, 100) *m/z* (positive ion mode)
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (90:10) Single spot observed, R_f = 0.15. Visualisation with UV at 254 nm
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellett
	Peaks:	3360, 1617, 1445, 1296, 1149, 1102, 937 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Key spectral data:	δ 0.66 (3H, s), 0.74 (3H, s), 1.07 (3H, s) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 11.5, 14.1, 20.4, 23.2, 25.8, 26.1, 28.6, 31.2, 31.4, 33.4, 35.9, 36.2, 38.3, 41.6, 45.0, 50.1, 53.3, 79.8, 97.9, 138.5, 158.8 ppm
Melting point:		298-306 °C
Microanalysis:	Found:	C = 73.0%; H = 9.2%; N = 8.2 % (April 1999)
	Calculated:	C = 73.2%; H = 9.4%; N = 8.1% (Calculated for $C_{21}H_{32}N_2O_2$)