Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

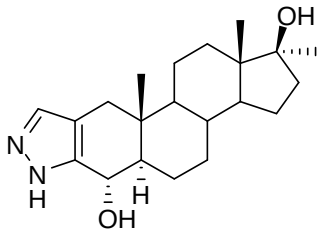
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D642: 4α-Hydroxystanozolol

Report ID: D642.2023.01 (Ampouled 220113)

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

Molecular Weight: 344.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
00-S-08	100356-20-5	946 ± 32 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,6S,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 February 2033, i.e. ten 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D642. The material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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 946 ± 32 μg of anhydrous 4α-hydroxystanozolol. The mass of analyte in each ampoule is calculated from the assigned indicative 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.

Stability: At the recommended storage conditions this ampouled material has demonstrated stability for a period of 10-15 years. The measurement uncertainty includes components for long term stability at the recommended storage conditions, and accelerated stability trials conducted at 40 ºC and 75% humidity for a 14 day period.

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/ELS 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.
6 March 2023

This report supersedes any issued prior to 06 March 2023.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	Alltech Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Methanol 0-6 min 63% B; 6-11 min 63-80% B; 11-12 min 80%B; 12-13 min 80-63%B; 13-20 min 63%B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (7 ampoules in duplicate, March 2022)
	Detector:	Shimadzu SPD-M20A PDA operating at 226 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 97.8%, s = 0.02% (7 ampoules in duplicate, March 2022)
	Re-analysis:	Mean = 97.8%, s = 0.03% (5 sub samples in duplicate, February 2023)

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

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The indicative purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV and evaporative light scattering (ELS) detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The indicative purity value was 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 elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler
	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 ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (3 sub samples in duplicate, May 2003)
	Re-analysis:	Mean = 99.9%, s = 0.02% (2 sub samples in duplicate, June 2004)
	Re-analysis:	Mean = 99.8%, s = 0.02% (7 sub samples in duplicate, September 2021)
	Detector:	UV at 226 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean > 98% (1 sub sample, March 2000)
	Re analysis:	Mean = 98.0%, s = 0.04% (3 sub samples in duplicate, May 2003)
	Re analysis:	Mean = 97.9%, s = 0.3% (2 sub samples in duplicate, June 2004)
	Re analysis:	Mean = 97.9%, s = 0.07% (7 sub samples in duplicate, September 2021)
Karl Fischer analysis:		Moisture content 3.7% mass fraction (November 2006) Moisture content 4.6% mass fraction (September 2021)
Thermogravimetric analysis:		Volatiles content 2.0% and non-volatile residue < 0.2% mass fraction (March 2000) Volatile content 2.3% (June 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 0.22 μm
	Program:	180 °C (0.5 min), 12 °C/min to 310 °C (3 min)
	Injector:	260 °C
	Split ratio:	30/1
	Transfer line temp:	300 °C
	Carrier:	Helium, 1mL/min
	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 (9.07 min):	560 (M^+, 40), 545 (39), 471 (14), 254 (56), 143 (100), 73 (85) *m/z*
ESI-MS:	Instrument:	Finnigan MAT TSQ 700 with electrospray interface.
	Operation:	Negative ion mode and positive ion mode, direct infusion at 5μL/min
	Ionisation:	ESI spray voltage 2.5 kV for -ve ion mode, 4.5kV for +ve ion mode
	Peak:	403.4 (MCH_3COO^-, 100), 343.3 (M^-), 325.0 ($M^- -H_2O$) *m/z* (-ve ion mode) 345.3 (MH^+, 100), 327.3 (MH^+-H_2O), 309.3 (MH^+-2H_2O) *m/z* (+ve ion mode)
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (90:10) Single spot observed, R_f = 0.3 (3 samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS-3000MX
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3290 (br), 1447, 1372, 1293, 1156, 1067, 947 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX.-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.67 (3H, s), 0.74 (3H, s), 1.07 (3H, s), 4.04 (1H, s) 7.19 (1H, br s) ppm
^{13}C NMR:	Instrument:	Bruker ARX-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 12.4, 14.1, 20.1, 23.2, 24.3, 26.1, 31.0, 31.4, 34.4, 35.9, 38.4, 45.0, 50.1, 51.0, 53.4, 65.7, 79.7, 113.6, 136.3, 139.7 ppm One signal obscured by the solvent peak
Melting point:		172 °C
Microanalysis:	Found:	C = 70.1%; H = 9.2%; N = 7.6% (April 2000)
	Calculated:	C = 73.2%; H = 9.4%; N = 8.1% (Calculated for $C_{21}H_{32}N_2O_2$)