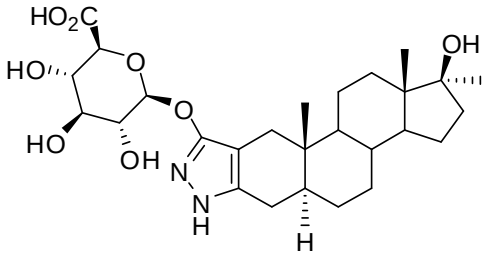
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D640: 3’-Hydroxystanozolol glucuronic acid



Report ID: D640.2025.01 (Ampouled 110307)

Chemical Formula: $C_{27}H_{40}N_2O_8$

Molecular Weight: 520.6 g/mol

Property value

Batch No.	CAS No.	Estimated mass per ampoule
00-S-14	361432-41-9	741 ± 35 µg

Synonym: 3′, 17β-Dihydroxy-17α-methyl-5α-androstano-[3,2-c] pyrazole 3′-β-glucuronide dihydrate

Expiration of certification: The property values are valid till 13 June 2028, 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 under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D640. Material was sourced from an external supplier, then certified for identity and purity by NMI Australia.

Intended use: This reference material should be used for qualitative analysis only. It is not suitable for calibration.

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 741 ± 35 µg of anhydrous 3’-hydroxystanozolol glucuronic acid. The mass of analyte in each ampoule is calculated from the assigned 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: This material has shown signs of consistent degradation since being ampouled in 2011. The bulk material used in the ampouling process had a purity of 99.96% by HPLC-UV, which has since decreased to 88.6%. The major impurity, currently estimated at 7.8%, has been identified as 3’-hydroxystanozolol. The material has also shown signs of decomposition in solution, as evidenced by duplicate analysis by HPLC-UV using samples dissolved in the mobile phase.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on five randomly selected ampoules of the material. The ampouled material does not show sufficient homogeneity at this level of sampling as the variation in analysis results between samples is significantly different at the 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.
31 July 2025

This report supersedes any issued prior to 31 July 2025.

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.

Characterisation Report:

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler OR Shimadzu Binary pump LC-20AB with SIL-20 A HT autosampler.
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/Milli Q water (55:45) 0.05% TFA was present in both aqueous and organic phases
.	Flow rate:	1.0 mL/min
	Detector:	PDA 2998 at 224 nm and ELSD 2420/Shimadzu LT-II ELSD

Relative peak area of the main component using ELSD

Initial analysis:	Mean = 98.3%, s = 0.17% (7 ampoules in duplicate, March 2011)
Re-analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, March 2012)
Re-analysis:	Mean = 96.5%, s = 0.21% (5 ampoules in duplicate, March 2015)
Re-analysis:	Mean = 97.6%, s = 0.05% (5 ampoules in duplicate, November 2020)
Re-analysis:	Mean = 98.4%, s = 0.05% (5 ampoules in duplicate, May 2023)

Relative peak area response of main component using UV at 224 nm:

Initial analysis:	Mean = 98.2%, s = 0.08% (7 ampoules in duplicate, March 2011)
Re-analysis:	Mean = 96.6%, s = 0.1% (5 ampoules in duplicate, March 2012)
Re-analysis:	Mean = 93.23%, s = 0.08% (5 ampoules in duplicate, March 2015)
Re-analysis:	Mean = 93.0%, s = 0.08% (5 ampoules in duplicate, February 2018)
Re-analysis:	Mean = 91.4%, s = 0.14% (5 ampoules in duplicate, November 2020)
Re-analysis:	Mean = 89.7%, s = 0.05% (5 ampoules in duplicate, May 2023)
Re-analysis:	Mean = 88.6%, s = 0.05% (2 ampoules in duplicate, June 2025)

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 quantitative nuclear magnetic resonance (qNMR). The three-proton singlet at 0.67 ppm was measured against a certified internal standard of maleic acid. Supporting evidence is provided by HPLC with UV/ELS detection, thermogravimetric analysis, Karl Fischer analysis, elemental microanalysis and ^{1}H NMR.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/Milli Q water (55:45) 0.05% TFA was present in both aqueous and organic phases
	Flow rate:	1.0 mL/min.
	Detector:	PDA 2998 at 224 nm and ELSD 2420

Relative peak area response of main component using ELSD:

Initial analysis:	Mean = 100% (3 sub samples in duplicate, May 2000)
Re-analysis:	Mean = 100% (2 sub samples in duplicate, June 2003)
Re-analysis:	Mean = 100% (2 sub samples in duplicate, November 2004)
Re-analysis:	Mean = 98.6%, s = 0.14% (5 sub samples in duplicate, March 2011)
Re-analysis:	Mean = 99.8%, s = 0.03% (3 sub samples in duplicate, March 2012)
Re-analysis:	Mean = 99.4%, s = 0.32% (3 sub samples in duplicate, March 2015)

Relative peak area response of main component using UV at 224 nm:

Initial analysis:	Mean = 99.9% (3 sub samples in duplicate, May 2000)
Re-analysis:	Mean = 100% (2 sub samples in duplicate, June 2003)
Re-analysis:	Mean = 100% (2 sub samples in duplicate, November 2004)
Re-analysis:	Mean = 100%, s = 0.02% (5 sub samples in duplicate, March 2011)
Re-analysis:	Mean = 99.1%, s = 0.02% (3 sub samples in duplicate, March 2012)
Re-analysis:	Mean = 98.8%, s = 0.14% (3 sub samples in duplicate, March 2015)

Karl Fischer analysis:	Moisture content 10.5% mass fraction (February 2007) Moisture content 9.1% mass fraction (May 2007) Moisture content 8.1% mass fraction (March 2007)
Thermogravimetric analysis:	Non volatile residue 1.6 % mass fraction (March 2011)

QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Initial analysis:	Mean (0.67 ppm) = 82.6%, s = 0.53% (3 sub samples, March 2007)
	Re-analysis:	Mean (0.67 ppm) = 83.5%, s = 0.18% (5 sub samples in duplicate, March 2011)

Spectroscopic and other characterisation data

FAB-MS:	Ions:	521$(MH)^+$, 345
	Ionisation:	15 kV in glycerol/H_20
HRMS:		Found m/z 521.2844; $C_{27}H_{41}N_2O_8$ (MH^+) requires m/z 521.2863
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/methanol/AcOH (67:30:3) Single spot observed, R_f = 0.1-0.2.
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, Nujol mull
	Peaks:	3342, 1725, 1637, 1602, 1519, 1455, 1372, 1078, 1014, 932 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance - 300
	Field strength:	300 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.80 (3H, s), 0.91 (3H, s), 1.24 (3H, s), 3.97 (1H, d), 5.13 (1H, d) ppm
	^{13}C NMR: Instrument:	Bruker Avance - 300
	Field strength:	75 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 11.9, 14.6, 21.9, 24.4, 26.1, 26.9, 30.2, 32.8, 32.9, 34.7, 37.5, 38.0, 39.3, 43.3, 46.7, 52.1, 55.3, 73.0, 74.5, 76.6, 77.4, 82.3, 101.6, 102.1, 141.3, 160.2, 172.2 ppm
Melting point:		200 °C (decomp)
Microanalysis:	Found:	C = 57.8%; H = 7.9%; N = 5.1% (May 2000)
	Found:	C = 56.5%; H = 7.9%; N = 4.9% (Dec 2006)
	Calculated:	C = 58.3%; H = 8.0%; N = 5.0% (Dihydrate) (Calculated for $C_{27}H_{40}N_2O_8$. $2H_2O$)