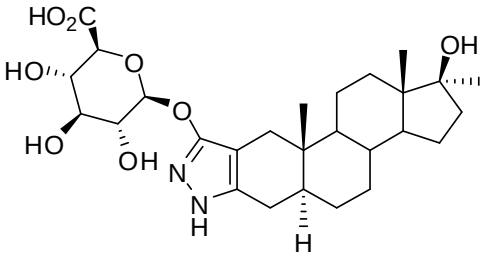
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D640: 3'-Hydroxystanozolol glucuronide



Report ID: D640.2018.02 (Ampouled 110307)

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

Molecular Weight: 520.6 g/mol

Property value

Batch No.	CAS No.	Mass per ampoule
00-S-14	**361432-41-9**	**792 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 12 February 2021, 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 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, 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 792 μg of anhydrous 3'-Hydroxystanozolol glucuronide. 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: The long-term stability of the compound in solution has not been examined.

HPLC with UV/ELSdetection**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.
17 March 2020

Characterisation Report:

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/MilliQ 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 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)
		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)

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 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/MilliQ 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 = 0.15
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}
^1H 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 $C_{27}H_{40}N_2O_8$. $2H_2O$)