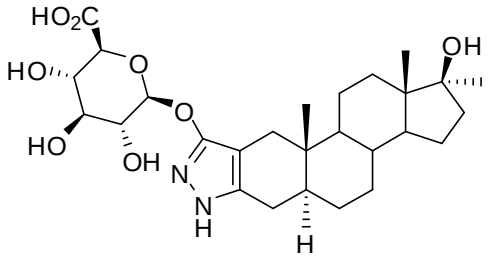
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D640.2018.01 (Ampouled 110307)
This batch of ampoules was prepared from the bulk material on 7th March 2011.

Compound Name: **3′-Hydroxystanozolol glucuronide**
Collection Number: D640
Chemical Formula: $C_{27}H_{40}N_2O_8$
CAS Registry Number: 361432-41-9
Structure:

Description: Off-white crystalline solid
Batch Number: 00-S-14
Molecular Weight: 520.6
Release date: May 2000
Metabolite of stanozolol



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

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 D640. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 792 µg of anhydrous 3′-hydroxystanozolol glucuronide.

The stated purity of 3′-hydroxystanozolol glucuronide in this material is indicative only. This material is recommended for qualitative analysis only.

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 = 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.

Purity estimate obtained by quantitative nuclear magnetic resonance (QNMR) using 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.

QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: d_6-DMSO
	Internal standard:	Potassium hydrogen maleate (98.8% m/m)
	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)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Waters XBridge C-18, 5µm (4.6 mm × 150 mm)
	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 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)
Thermogravimetric analysis:		Non volatile residue 1.6 % mass fraction (March 2011)
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)

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}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz Solvent: CD_3OD (3.31 ppm)
	Key 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 Advance-300
	Field strength:	75 MHz Solvent: CD_3OD (49.00 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) Calc: C = 58.3%; H = 8.0%; N = 5.0% (Dihydrate) (Calculated for $C_{27}H_{40}N_2O_8$. $2H_2O$)

Expiration of certification
The property values are valid till 12th 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.

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

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years.

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: 15 February, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 15th February 2018.

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