Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

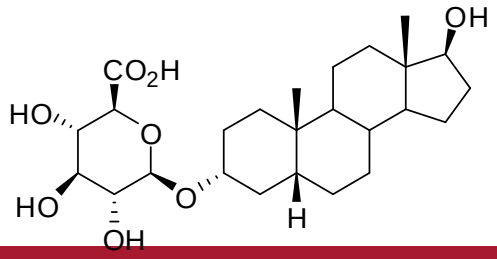
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S003b: 5β-Androstane-3α, 17β-diol-3-O-β-glucuronic acid

Report ID: S003b.2019.01

Chemical Formula: $C_{25}H_{40}O_8$

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
16-S-03	114761-91-0	94.7 ± 1.9 %

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (3α,5β,17β)-17-Hydroxyandrostan-3-yl β-D-glucopyranosiduronic acid

Expiration of certification: The property values are valid till 19 June 2022, 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 sample bottles 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: Off-white powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: 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. 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 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.
25 June 2019

This report supersedes any issued prior to 25 June 2019

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value by qNMR was obtained using the doublet at 4.6 ppm measured against a certified internal standard of potassium hydrogen maleate. The certified purity value by qNMR was subsequently corrected by the results Karl Fischer analysis.

Supporting evidence is provided by traditional analytical techniques HPLC with evaporative light scattering detection, thermogravimetric analysis, ^{1}H NMR spectroscopy and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	Methanol/MilliQ water (60:40)
		Formic acid (0.2% v/v) was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 100.0%, s = 0.01% (7 sub samples in duplicate, March 2016)
	Re-analysis:	Mean = 99.9%, s = 0.05% (5 sub samples in duplicate, April 2018)
Thermogravimetric analysis:		Volatile content 3.5% and non volatile residue < 0.2% mass fraction (March 2016).
Karl Fischer analysis:		Moisture content 5.0% mass fraction (January 2017)
		Moisture content 4.7% mass fraction (January 2018)
		Moisture content 4.0% mass fraction (June 2019)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	AcOH-d_4 (2.07 ppm)
	Internal standard:	Potassium hydrogen maleate (99.6% mass fraction)
	Initial analysis:	Mean (4.6 ppm) = 93.7%, s = 0.1% (3 sub samples, January 2017)

Spectroscopic and other characterisation data

GC-MS:	The free steroid was liberated upon treatment with β-glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA.	
	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 30 °C /min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium
	Split ratio:	20/1
	The retention time of the *bis*-TMS derivative of 5β-androstan-3α, 17β-diol is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Bis-TMS (8.8min):	436 (M+, 4), 421 (8), 346 (27), 256 (79), 241 (73), 215 (49), 201 (29), 199 (27), 161 (119), 147 (23), 129 (89), 107 (25), 93 (27), 81 (26), 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F254. Chloroform/methanol (2/1) Single spot observed, Rf = 0.25. Visualisation with vanillin
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3446, 3344, 2939, 2896, 1719, 1457, 1449, 1375, 1259, 1213, 1161, 1122, 1042, 999, 696 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.96 (3H, s), 0.97-1.18 (4H, m), 1.19-1.52 (10H, m), 1.54-1.64 (2H, m), 1.76-2.03 (5H, m), 1.98 (1H, m), 3.19 (1H, dd, *J* = 8.1, 9.0 Hz), 3.38 (1H, t, *J* = 9.2 Hz), 3.52 (1H, t, *J* =9.5 Hz), 3.57 (1H, t, *J* = 8.6 Hz), 3.69 (1H, m), 3.78 (1H, d, *J* = 9.7 Hz), 4.45 (1H, d, *J* = 7.7 Hz) ppm
		Two related impurities estimated at 0.5% and 0.2% mass fraction was observed in the ^{1}H NMR (AcOH-d_4)
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 11.6, 21.5, 23.9, 24.3, 27.2, 27.7, 28.2, 30.7, 35.3, 35.9, 36.3, 37.3, 38.2, 42.0, 43.6, 44.2, 52.4, 73.2, 74.8, 76.6, 77.5, 80.3, 82.6, 102.6, 172.7 ppm
Melting point:		210-211 ºC
Microanalysis:	Found:	C = 61.5%; H = 8.8% (April 2016)
	Calculated:	C = 61.3%; H = 8.7% (Calculated for $C_{25}H_{40}O_8$ with 4.3% water)