



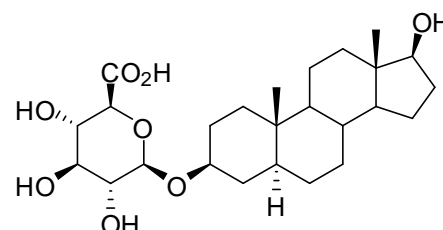
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D678b: 5 α -Androstane-3 β , 17 β -diol-3-O- β -glucuronic acid

Report ID: D678b.2023.01 (Ampouled 200709)

Chemical Formula: C₂₅H₄₀O₈

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
14-S-08	1569283-12-0	901 ± 39 μ g

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

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

Expiration of certification: The property values are valid till 22 March 2026, 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 CRM is intended for a single use to prepare a standard solution containing D678b. This material was prepared by synthesis, and certified for identity and purity by NMIA.

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

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. 2-Propanol). This will transfer 901 ± 39 μ g of anhydrous 5 α -androstane-3 β , 17 β -diol-3-O- β -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 °C 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: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 evaporative light scattering 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.
14 April 2023

This report supersedes any issued prior to 14 April 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.

Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler
	Column:	X-Bridge C-18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Milli-Q water/methanol (45:55)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.04% (7 ampoules in duplicate, September 2020)
	Re-analysis:	Mean = 99.6%, s = 0.11% (5 ampoules in duplicate, March 2023)
	HPLC:	Instrument:
Column:		X-Bridge C-18, 5 μ m (4.6 mm x 150 mm)
Column oven:		40 $^{\circ}$ C
Mobile Phase:		Milli-Q water/methanol (45:55)
Flow rate:		1.0 mL/min
Detector:		Dionex Charged Aerosol Detector
Relative peak area of the main component:		
Initial analysis:		Mean = 97.5%, s = 0.08% (7 ampoules in duplicate, August 2020)
Re-analysis:		Mean = 97.4%, s = 0.10% (5 sub samples in duplicate, September 2021)
Re-analysis:		Mean = 97.2%, s = 0.13% (5 ampoules in duplicate, March 2023)

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 certified purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The one-proton doublet at 4.6 ppm was measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by a combination of traditional analytical techniques, including HPLC with ELS and charged aerosol detection, thermogravimetric analysis, Karl Fischer analysis, 1 H NMR spectroscopy and qualitative elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler Or Waters Binary pump 1525, 717 auto sampler	
	Column:	X-Bridge, 5 μ m (4.6 mm x 150 mm)	
	Column oven:	40 $^{\circ}$ C	
	Mobile Phase:	Milli-Q water/methanol (45:55) The aqueous phase contained 0.1% v/v formic acid.	
	Flow rate:	1 mL/min	
	Detector:	Shimadzu ELSD-LT II or Waters ELSD 2424	
	Relative peak area of the main component:		
	Initial analysis:	Mean = 99.6%, s = 0.09% (9 sub samples in duplicate, May 2015)	
	Re-analysis:	Mean = 99.5%, s = 0.10% (5 sub samples in duplicate, April 2016)	
	Re-analysis:	Mean = 99.8%, s = 0.06% (5 sub samples in duplicate, May 2017)	
Re-analysis:	Mean = 99.7%, s = 0.08% (5 sub samples in duplicate, April 2020)		
HPLC:	Instrument:	Thermo Scientific UltiMate 3000	
	Column:	X-Bridge, 5 μ m (4.6 mm x 150 mm)	
	Column oven:	40 $^{\circ}$ C	
	Mobile Phase:	Milli-Q water/methanol (45:55) The aqueous phase contained 0.1% v/v formic acid.	
	Flow rate:	1 mL/min	
	Detector:	Dionex Charged Aerosol Detector	
	Relative peak area of the main component:		
	Initial analysis:	Mean = 99.7%, s = 0.05% (7 sub samples in duplicate, May 2020)	
	Karl Fischer analysis:	Moisture content 9.1% mass fraction (May 2015)	
		Moisture content 10.4% mass fraction (April 2016)	
Moisture content 10.9% mass fraction (May 2017)			
Moisture content 9.2% mass fraction (March 2020)			
Thermogravimetric analysis:	Non volatile residue < 0.2% mass fraction (May 2015)		
	The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis		
qNMR:	Instrument:	Bruker Avance-III-500	
	Field strength:	500 MHz	
	Solvent:	AcOH- d_4 (2.06 ppm)	
	Internal standard:	Potassium hydrogen maleate (99.7% mass fraction)	
	Initial analysis:	Mean (4.6 ppm) = 90.5%, s = 0.1% (2 sub samples, June 2020)	

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 10 μ L/min
	Ionisation:	ESI spray voltage at 3.5 kV negative ion
	EM voltage:	650 V
	Cone voltage:	10 V
	Peak:	467.4 [M-H] ⁻ <i>m/z</i>
IR:	Instrument:	Biorad FT-IR
	Range:	4000-400 cm^{-1} , neat.
	Peaks:	3323, 2927, 2848, 1720, 1447, 1043, 1023 cm^{-1}
¹ H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 0.66 (1H, m), 0.72 (3H, s), 0.85 (3H, s), 0.89-1.06 (4H, m), 1.12 (1H, m), 1.20-1.61 (10H, m), 1.68-1.71 (2H, m), 1.74 (1H, ddd, <i>J</i> = 3.6, 3.6, 13.3 Hz), 1.81 (1H, ddd, <i>J</i> = 3.1, 3.3, 12.4 Hz), 1.87 (1H, m), 1.96 (1H, m), 3.18 (1H, dd, <i>J</i> = 7.9, 9.2 Hz), 3.37 (1H, t, <i>J</i> = 9.2 Hz), 3.51 (1H, t, <i>J</i> = 9.5 Hz), 3.55 (1H, t, <i>J</i> = 8.5 Hz), 3.65 (1H, m), 3.78 (1H, d, <i>J</i> = 9.7 Hz), 4.44 (1H, d, <i>J</i> = 7.8 Hz) ppm
¹³ C NMR:	Instrument:	Bruker DMX600
	Field strength:	150 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49 ppm)
	Spectral data:	δ 10.7, 11.7, 21.0, 23.3, 28.9, 29.4, 29.6, 31.9, 34.4, 35.8, 35.9, 37.1, 37.3, 43.1, 45.1, 51.4, 55.0, 72.2, 73.8, 75.6, 76.5, 78.8, 81.5, 101.7, 171.7 ppm
Melting point:		205 $^{\circ}$ C (dec.)
Microanalysis:	Found:	C = 58.0%; H = 8.6% (May, 2015)
	Calculated:	C = 58.3%; H = 8.8% (Calculated for C ₂₅ H ₄₀ O ₈ + 9.1% H ₂ O)