



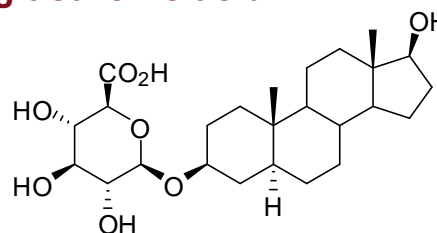
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

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

Report ID: D678b.2020.01

Chemical Formula: C₂₅H₄₀O₈

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
14-S-08	1569283-12-0	90.5 ± 1.5%

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 3 June 2025, i.e. five 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.

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 °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 five 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 1-2 mg sub samples 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 a 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.
23 June 2020

This report supersedes any issued prior to 23 June 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

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, ¹H NMR spectroscopy and qualitative elemental microanalysis.

HPLC:

Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
Or Waters Binary pump 1525, 717 autosampler

Column: X-Bridge, 5 μ m (4.6 mm x 150 mm)

Column oven: 40 °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 °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 °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)