



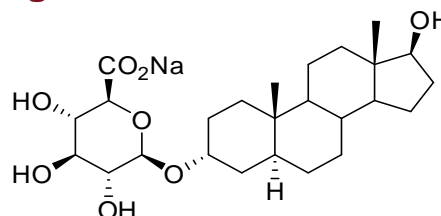
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S051: 5 α -Androstane-3 α , 17 β -diol-3-O- β -glucuronide sodium salt

Report ID: S051.2023.01

Chemical Formula: C₂₅H₃₉NaO₈

Molecular Weight: 490.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
20-S-04	65535-18-4 (free acid)	78.3 \pm 1.9%

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

IUPAC name: Sodium (3 α ,5 α ,17 β)-17-hydroxyandrostane-3-yl β -D-glucopyranosiduronate.

Expiration of certification: The property values are valid till 20 September 2026, 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: 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.

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. 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 long term 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 ten years. The measurement uncertainty at the 95% confidence interval also includes a stability component determined from accelerated stability trials conducted at 40 °C and 75% humidity for a 14 day period.

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 CAD 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.
11 October 2023

This report supersedes any issued prior to 11 October 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:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value by quantitative NMR was obtained using the one-proton doublet at 4.55 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by Karl Fisher analysis of water, HPLC with charged aerosol detection (CAD), and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	d ₄ -AcOH (2.05 ppm)
	Internal standard:	Maleic acid (98.8% mass fraction)
	Initial analysis:	Mean (4.55 ppm) = 77.7%, s = 0.4% (5 sub samples, March 2021)
HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Alltima C-18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water pH 2.3 by formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS charged aerosol detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 95.7%, s = 0.2% (7 sub samples in duplicate, September 2021)
	Re-analysis:	Mean = 95.8%, s = 0.1% (7 sub samples in duplicate, September 2023)
Karl Fischer analysis:		Moisture content 17.7% mass fraction (January 2021)
		Moisture content 17.1% mass fraction (September 2023)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 20 μ L/min
	Ionisation:	ESI spray voltage at 2.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	467 (M-Na ⁺) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Chloroform/methanol (2:1) Single spot observed, R _f = 0.3-0.4. Vanillin.
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm ⁻¹ , neat
	Peaks:	3327, 2930, 1606, 1409, 1018 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 0.72 (3H, s), 0.79 (1H, m), 0.83 (3H, s), 0.86-1.04 (3H, m), 1.12-1.71 (15H, m), 1.78-1.88 (2H, m), 1.96 (1H, m), 3.23 (1H, t, <i>J</i> = 8.3 Hz), 3.39 (1H, t, <i>J</i> = 8.8 Hz), 3.44 (1H, t, <i>J</i> = 9.0 Hz), 3.53 (1H, d, <i>J</i> = 9.4 Hz), 3.55 (1H, t, <i>J</i> = 8.6 Hz), 4.06 (1H, m), 4.33 (1H, d, <i>J</i> = 7.8 Hz) ppm
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49 ppm)
	Spectral data:	δ 11.7, 11.9, 21.5, 24.3, 25.8, 29.6, 30.6, 32.8, 33.6, 35.5, 36.9, 37.0, 38.2, 40.6, 44.1, 52.6, 55.7, 73.8, 74.2, 75.0, 76.3, 78.0, 82.6, 102.0, 177.0 ppm Androsterone glucuronide sodium salt was estimated at 1.2% mass fraction by ¹ H NMR acquired under qNMR conditions.
Melting point:		230 °C decomposes
Microanalysis:	Found:	C = 48.8%; H = 8.1% (February 2021)
	Calculated:	C = 61.2%; H = 8.0% (Calculated for C ₂₅ H ₃₉ NaO ₈)
	Calculated:	C = 48.7%; H = 8.7% (Calculated for C ₂₅ H ₃₉ NaO ₈ (H ₂ O) ₇)