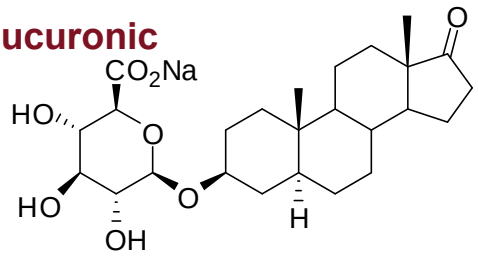
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S031: 5α-Androstane-3β-ol-17-one-3-O-β-glucuronic acid, sodium salt



Report ID: S031.2019.01

Chemical Formula: $C_{25}H_{37}NaO_8$

Molecular Weight: 488.6 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
15-S-04	**21085-78-9**	**79.6 ± 3.5%**

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

Synonyms: Epi-androsterone glucuronide, sodium salt
17-Oxo-5α-androstan-3β-yl glucopyranosiduronic acid, monosodium salt

Expiration of certification: The property values are valid till 18 November 2024, 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 solid 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: 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: The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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 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.
11 December 2019

This report supersedes any issued prior to 11 December 2019

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) using a combination of the one proton doublet at 4.19 ppm against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided HPLC with ELS detection, Karl Fischer analysis, ^{1}H NMR and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	MilliQ water/methanol (45:55)
		0.1% Formic acid was present in the aqueous phase
	Flow rate:	1 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.12% (9 sub samples in duplicate, May 2015)
Karl Fischer analysis:		Moisture content 16.7% mass fraction (May 2015)
		Moisture content 19.5% mass fraction (November 2019)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Potassium hydrogen maleate (100% mass fraction)
	Initial analysis:	Mean (4.19 ppm) = 82.8%, s = 0.6% (5 sub samples, May 2015)
	Initial analysis:	Mean (4.19 ppm) = 82.4%, s = 0.4% (5 sub samples, May 2015, spectra reprocessed November 2019)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 10 µL/min
	Ionisation:	ESI spray voltage at 3.5 kV negative ion
	EM voltage:	650 V
	Cone voltage:	10 V
	Peak:	465.4 ($M\text{-}H^+$) *m/z*
IR:	Instrument:	Biorad FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3281 (br), 2911, 2845, 1726, 1593, 1414, 1035, 1011 cm^{-1}
^1H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.67 (1H, m), 0.77 (3H, s), 0.79 (3H, s), 0.88-0.99 (2H, m), 1.04 (1H, m), 1.09-1.18 (2H, m), 1.19-1.33 (4H, m), 1.34-1.54 (3H, m), 1.54-1.69 (4H, m), 1.73 (1H, m), 1.76-1.87 (2H, m), 1.99 (1H, ddd, *J* = 9.0, 9.0, 18.4 Hz), 2.36 (1H, dd, *J* = 8.5, 19.0 Hz), 2.87 (1H, t, *J* = 8.3 Hz), 3.02 (1H, dd, *J* = 8.8, 9.8 Hz), 3.09 (1H, ddd, *J* = 4.1, 8.8, 8.8 Hz), 3.14 (1H, d, *J* = 9.9 Hz), 3.55 (1H, m), 4.19 (1H, d, *J* = 7.8 Hz), 4.82 (1H, br s), 4.86 (1H, d, *J* = 4.4 Hz), 7.00 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 12.1, 13.5, 20.1, 21.4, 28.2, 29.0, 30.5, 31.4, 34.0, 34.5, 35.36, 35.42, 36.6, 44.1, 47.1, 50.6, 53.8, 72.3, 73.4, 73.6, 76.0, 76.9, 100.3, 172.7, 219.9 ppm
Microanalysis:	Found:	C = 50.9%; H = 8.1% (March, 2015)
	Calculated:	C = 50.9%; H = 8.2% (Calculated for $C_{25}H_{37}NaO_8$ + 17.1% water)