Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

CIPM MRA

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

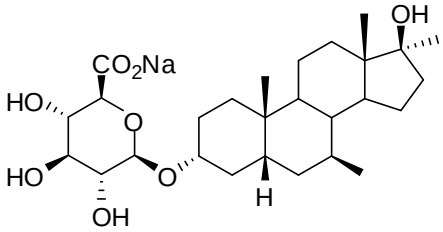
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D629: 7β,17α-Dimethyl-5β-androstan-3α,17β-diol-3-O-β-glucuronide (Na salt)

Report ID: D629.2020.01

Chemical Formula: $C_{27}H_{43}O_8Na$

Molecular Weight: 518.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
00-S-03	362499-08-9 (free acid)	84.1 ± 1.3%

IUPAC name: Sodium (3α,5β,7β,17β)-17-Hydroxy-7,17-dimethylandrostan-3-yl β-*D*-glucopyranosiduronate.

Expiration of certification: The property values are valid till 14 January 2023, 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.

Description: White amorphous powder sourced from an external supplier, 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%.

Stability: This material has demonstrated stability over a minimum period of five 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 ELSD detection on six 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.
29 January 2020

This report supersedes any issued prior to 29 January 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 mass balance from a combination of traditional analytical techniques, including HPLC with evaporative light scattering and charged aerosol detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

HPLC:	Instrument:	Waters Alliance 2650
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = 10mM ammonium acetate pH 4.2; B = Acetonitrile 0-8 min 35% B; 8-9 min 35-80% B; 9-13 min 80%B; 13-14 min 80-35%B.
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.05% (5 sub samples in duplicate, January 2020)
HPLC:	Instrument:	Thermo Scientific Ultimate 3000 RS Pump, RS Autosample, RS Column Compartment
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = 10mM ammonium acetate pH 4.2; B = Acetonitrile 0-8 min 35% B; 8-9 min 35-80% B; 9-13 min 80%B; 13-14 min 80-35%B.
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS CAD Detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (5 sub samples in duplicate, January 2020)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/10mM ammonium acetate pH 4.2 (30:70 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424 or Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (6 sub samples in duplicate, September 2006)
	Re-analysis:	Mean = 99.9%, s = 0.02% (5 sub samples in duplicate, September 2009)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, August 2014)
Karl Fischer analysis:		Moisture content 12.5% mass fraction (August 2006 & September 2009) Moisture content 14.1% mass fraction (August 2014) Moisture content 15.7% mass fraction (January 2020)
Thermogravimetric analysis:		Volatiles content 12% mass fraction (August 2006)

Spectroscopic and other characterisation data

LC-MS:	Peak area percentage of total > 95% of organic component	
	Instrument:	Perkin-Elmer Sciex API 300
	Column:	Phenomenex LUNA C18 5 μm (1 mm × 150 mm)
	Eluent:	A: 15 mM ammonium acetate, pH 4.2: methanol (9:1) B: Methanol: 15 mM ammonium acetate, pH 4.2 (9:1)
	Gradient:	40% B to 90% B in 15 min
	Flow Rate:	100 μL/min, post column split 1:10
	The retention time is reported with the major peaks observed in the positive ion mass spectrum. The latter are reported in mass/charge ratios with (in brackets) their assignment and as a percentage relative to the base peak.	
	14.8 min:	519 ($[M-Na]^+$, 26), 514 ($[M-NH_4]^+$, 100), 497 ($[M-H]^+$, 4).
ESI-MS:	Instrument	Perkin-Elmer Sciex API 300
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH_4OAc, pH 4.2: MeOH (1:1).
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range m/z 100-600.
	Major ions:	541 (59), 519 (98), 514(45), 497 (3) *m/z*
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH_4OAc: MeOH (1:1).
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range *m/z* 100-600.
	Major ions:	495 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol/water (1:1) Single spot observed, R_f = 0.2
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3400, 1605 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	D_2O
	Spectral data:	δ 0.80 (3H, s), 0.88 (3H, d), 0.95 (3H, s), 1.18 (3H, s), 4.56 (1H, d) ppm Acetone was observed at 0.08% mass fraction in the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	D_2O
	Spectral data:	δ 14.8, 21.1, 23.7, 23.8, 25.3, 26.7, 27.3, 31.9, 32.1, 34.4, 34.6, 35.7, 38.2, 38.7, 40.7, 42.6, 44.4, 47.1, 50.8, 72.3, 73.5, 76.2, 76.8, 80.1, 81.7, 100.4, 176.0 ppm
HRMS:	Found	*m/z* 519.293; $C_{27}H_{44}O_8Na$ ($M-Na-H^+$) requires m/z 519.293 *m/z*.