Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

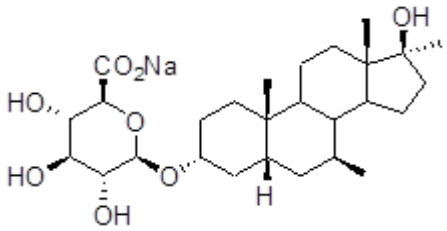
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

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

Report ID: D629.2024.01 (Ampouled 240110)

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

Molecular Weight: 518.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
00-S-03	362499-08-9 (free acid)	837 ± 43 μg

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

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 22 February 2027, three years from the date of 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. 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: 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 D629. This material was sourced from an external supplier 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. methanol). This will transfer 837 ± 43 μg of anhydrous 7β,17α-Dimethyl-5β-androstan-3α,17β-diol-3-O-β-glucuronide (Na salt). 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 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 all impurities are quantified as a mass fraction and subtracted from 100%.

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 ºC and 75% humidity for 14 days.

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 charged aerosol 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

Chemical Reference Materials, NMI.
14 June 2024

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:

UHPLC:	Instrument:	Thermo Scientific UltiMate 3000 RS Pump, RS Autosample, RS Column Compartment
	Column:	ACE, super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase	A = MilliQ water (10mM ammonium acetate, pH 4.2) B = Acetonitrile 0-15 min 35% B; 15-17 min 35-80% B; 17-20 min 80%B; 20-21 min 80-35%B.
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS CAD Detector
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 96.6%, s = 0.05% (7 ampoules in duplicate, February 2024)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE, super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water (10mM ammonium acetate, pH 4.2) B = Acetonitrile 0-12 min 35% B; 12-15 min 35-80% B; 15-17 min 80%B; 17-18 min 80-35%B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.97%, s = 0.01% (7 ampoules in duplicate, May 2024)

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 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.

Supporting evidence is provided by elemental microanalysis.

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)
UHPLC:	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)
	Re-analysis:	Mean = 98.5%, s = 0.14% (5 sub samples in duplicate, September 2022)
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) Moisture content 14.9% mass fraction (September 2022)
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 quantified 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*.