Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
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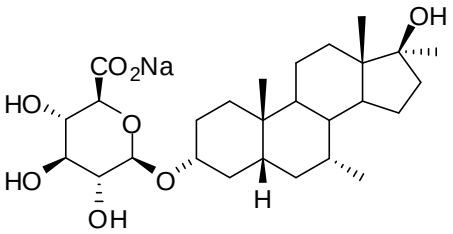
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D628: 7α,17α-Dimethyl-5β-androstan-3α,17β-diol-3-β-D-glucuronide (Na salt)

Report ID: D628.2020.01 (Ampouled 090730)

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

Molecular Weight: 518.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
00-S-02	362499-07-8 (free acid)	841 μg

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

Expiration of certification: The property values are valid till 29 May 2030, i.e. ten 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D628. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

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 841 μg of anhydrous 7α,17α-Dimethyl-5β-androstan-3α,17β-diol-3-β-D-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.

Stability: 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.
16 June 2020

This report supersedes any issued prior to 16 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:

HPLC:	Instrument:	Waters HPLC
	Column:	Alltech Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/ 10 mM ammonium acetate pH 4.2 (28:72 v/v)
	Flow rate:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.99%, s = 0.01% (7 ampoules in duplicate, August 2009)
	Re-analysis:	Mean = 99.92%, s = 0.00% (5 ampoules in duplicate, May 2012)
	Re-analysis:	Mean = 99.90%, s = 0.01% (5 ampoules in duplicate, May 2015)
	Re-analysis:	Mean = 100.00%, s = 0.00% (5 ampoules in duplicate, May 2020)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (28:72 v/v) A = 10 mM ammonium acetate pH 4.2 in MilliQ water; B = Acetonitrile
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2420
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, September 2006)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, August 2009)
Karl Fischer analysis:		Moisture content 13.3% mass fraction (August 2006) Moisture content 13.2% mass fraction (August 2009)
Thermogravimetric analysis:		Volatiles content 13.0% 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)
	Column temp:	45 °C
	Solvent system:	A: 15 mM ammonium acetate, pH 4.2: methanol B: Methanol (9:1)
	Gradient:	40% B to 90% B in 15 min
	Flow rate:	0.1 mL/min,post column split 1:10
	The retention time of 7α, 17α-dimethyl-5β-androstan-3α,17β-diol-3-β-D-glucuronide (Na salt) is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	13.4 min:	519 ($[MNa]^+$, 26), 514 ($[MNH_4]^+$, 100), 497 ($[MH]^+$, 3) *m/z*
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 (64), 519 (97), 514(49), 497 (2) *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
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3419, 1703 cm^{-1}
^1H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 0.79 (3H, s), 0.95 (3H, s), 0.98 (3H, d, *J* = 7.2 Hz), 1.19 (3H, s), 4.53 (1H, d, *J* = 8.1 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	D_2O
	Spectral data:	δ 13.9, 17.8, 20.4, 23.0, 23.3, 25.1, 27.1, 29.3, 31.5, 32.8, 34.7, 35.3, 35.7, 37.6, 37.9, 38.4, 42.7, 45.6, 45.9, 72.3, 73.4, 76.2, 76.7, 80.6, 82.9, 100.4, 176.1 ppm
Melting point:		227 °C (decomp)
HRMS:		Found m/z 519.292; $C_{27}H_{44}O_8Na$ ($MNaH^+$) requires *m/z* 519.293 *m/z*