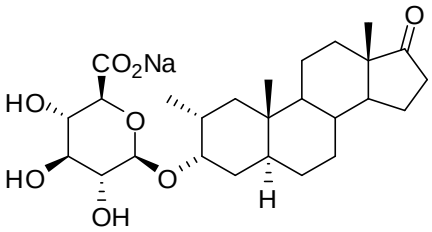
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D601: 2α-Methyl-5α-androstan-3α-ol-17-one-3β-D-glucuronide (Na salt)

Report ID: D601.2022.02 (Ampouled 100907)

Chemical Formula: $C_{26}H_{39}O_8Na$

Molecular Weight: 502.6 g/mol (base)



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-19	361432-78-2 (free acid)	906 ± 13 μg

IUPAC name: Sodium (2α, 3α, 5α)-3-hydroxy-2-methylandrostan-17-one-3-yl- β-D-glucopyranosiduronate.

Expiration of certification: The property values are valid till 24 May 2027, 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 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 RM is intended for a single use to prepare a standard solution containing D601. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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 approximately 906 μg of anhydrous 2α-methyl-5α-androstan-3α-ol-17-one-3β-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: This material has demonstrated stability over a minimum period of three 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 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.
2 November 2022

This report supersedes any issued prior to 2 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler or Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A: 20 mM ammonium acetate (pH 4.2), B: Acetonitrile, 0-7 min 33% B, 7-8 min 33% B-60% B, 8-13 min 60% B, 13-14 min 60% B-33% B, 14-20 min 33% B
	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% (7 ampoules in duplicate, September 2010)
	Re-analysis:	Mean = 100.0%, s = 0.01% (5 ampoules in duplicate, October 2011)
	Re-analysis:	Mean = 99.9%, s = 0.04% (5 ampoules in duplicate, October 2014)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, September 2017)
	Re-analysis:	Mean = 99.7%, s = 0.08% (5 ampoules in duplicate, June 2022)
HPLC:	Instrument:	SIL-20 A HT auto sampler Thermo Scientific UltiMate 3000
	Column:	X-Bridge C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A: 20 mM ammonium acetate (pH 4.2), B: Acetonitrile, 0-9 min 33% B, 9-10 min 33% B-60% B, 10-11 min 60% B, 11-16 min 60% B-33% B, 16-22 min 33% B
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS CAD Detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.06% (5 ampoules in duplicate, May 2022)

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 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.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/50 mM ammonium acetate (pH 4.2) [30:70] (2004) Acetonitrile/20 mM ammonium acetate (pH 4.2) [35:65] (2007)
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean > 99% (January 2000)
	Re-analysis:	Mean = 100%, s = 0% (3 sub samples in duplicate, 2004)
	Re-analysis:	Mean = 100%, s = 0% (5 sub samples in duplicate, 2007)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate (pH 4.2) [33:67]
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.98%, s = 0.002% (7 sub samples in duplicate, October 2010)
Karl Fischer analysis:		Moisture content 5.2% mass fraction (August 2006) Moisture content 5.3% mass fraction (September 2010)
Thermogravimetric analysis:		Volatile content 9.0% mass fraction (June 1999) Volatile content 8.4% mass fraction (September 2010)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Perkin-Elmer Sciex API 300
	Column:	Phenomenex LUNA C-18 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 of 2α-Methyl-5α-androstan-3α-ol-17-one-3β-D-glucuronide (Na salt) is reported along with the major peaks observed in the positive ion mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as percentage relative to the base peak.	
	16.6 min:	503 ($[M+Na]^+$, 22), 498 ($[M+NH_4]^+$, 100), 481 ($[M+H]^+$, 6), 463, 287 *m/z*
ESI-MS:	Instrument:	Perkin-Elmer Sciex API 300
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH4OAc, pH 4.2: MeOH (1:1)
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range of 100-600 *m/z*
	Major ions:	503 (100), 498 (41), 481 (3), 461 (10), 463, 445, 287, 269 *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 of 100-600 *m/z*
	Major ions:	479 ($[M-H]^-$, 16), 157 (100) *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3480, 3140, 1735, 1615, 1604, 1454, 1409, 1157, 1068, 1017 cm^{-1}
^1H NMR:	Instrument:	Bruker Advance-DMX-600
	Field strength:	300 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 0.80 (3H, s), 0.84 (3H, s), 0.96 (3H, d, *J* = 6.6 Hz), 3.76 (1H, br s), 4.41 (1H, d, *J* = 7.8 Hz) ppm
		Methanol estimated at 3.6% mass fraction (May 1999) Methanol estimated at 4.5% mass fraction (August 2006) Methanol estimated at 3.9% mass fraction (October 2010)
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 12.6, 14.1, 18.5, 20.4, 22.0, 28.0, 30.9, 31.6, 32.3, 35.2, 35.3, 36.3, 36.3, 39.4, 41.9, 48.8, 51.2, 54.3, 72.4, 74.0, 76.2, 76.7, 82.1, 104.3, 176.2, 228.3 ppm
Melting point:		229-231 °C
Microanalysis:	Found:	C = 58.3%; H = 8.3% (August 2006)
	Calculated:	C = 62.1%; H = 7.8% (Calculated for $C_{26}H_{39}O_8Na$)