Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

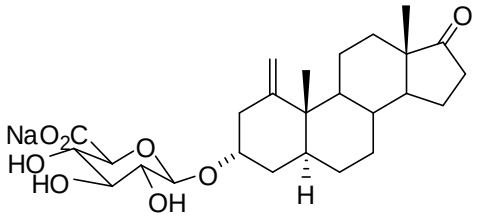
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D597: 1-Methylene-5α-androstan-3α-ol-17-one-3-β-D-glucuronide (sodium salt)

Report ID: D597.2019.02 (Ampouled 080922)

Chemical Formula: $C_{26}H_{37}O_8Na$

Molecular Weight: 500.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-15	**Not available**	**712 μg**

Synonym: Methenolone M1 β-D glucuronide (sodium salt)

Expiration of certification: The property values are valid till 8 July 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 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 D597. The 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 712 μg of anhydrous 1-methylene-5α-androstan-3α-ol-17-one-3-β-D-glucuronide (sodium 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 five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual 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 UV and 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.
17 February 2020

This report supersedes any issued prior to 17 February 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:	Column:	X-Bridge C-18 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = 0.05% TFA in MilliQ Water/B= 0.05 %TFA in MeOH (38 %: 62%)
	Flow Rate:	1.0 mL/min, Isocratic flow
	Detector:	Waters PDA 2998 operating at 203 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.2 %, s = 0.09% (7 ampoules in duplicate, September 2008)
	Re-analysis:	Mean = 99.7 %, s = 0.17% (5 ampoules in duplicate, September 2009)
	Re-analysis:	Mean = 99.4 %, s = 0.07% (5 ampoules in duplicate, July 2014)
	Re-analysis:	Mean = 99.4 %, s = 0.04% (5 ampoules in duplicate, July 2019)
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9 %, s = 0.016% (7 ampoules in duplicate, September 2008)
	Re-analysis:	Mean = 99.7 %, s = 0.01% (5 ampoules in duplicate, September 2009)

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 quantitative NMR against an internal standard of potassium hydrogen maleate. Supporting evidence is provided by HPLC, Karl Fischer analysis, thermogravimetric analysis and elemental microanalysis.

HPLC:	Column:	X-Bridge C-18 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = 0.05% TFA in MilliQ Water/B= 0.05 %TFA in MeOH (38 %: 62%)
	Flow Rate:	1.0 mL/min, Isocratic flow
	Detector:	Waters PDA 2998 operating at 203 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = > 99% (3 sub samples, January 2000)
	Re-analysis:	Mean = 99.2 %, s = 0.18 (5 sub samples in duplicate, September 2008)
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9 %, s = 0.008 (2 sub samples in duplicate, November 2004)
	Re-analysis:	Mean = 99.9 %, s = 0.013 (5 sub samples in duplicate, September 2008)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	D_2O
	Internal standard:	Potassium hydrogen maleate
	Purity estimate:	73.8% (mass fraction %, mean of five sub samples, s = 1.4%, October 2008)
Thermogravimetric analysis:		Volatiles content 8.8% mass fraction (September 2008)
Karl Fischer analysis:		Moisture content 10.9% mass fraction (September 2008)

Spectroscopic and other characterisation data

LC-MS:	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 as mass to charge ratio with (in brackets) their assignment and as a percentage relative to the base peak.	
	13.4 min:	501 ([M-Na]$^+$, 14), 496 ([M-NH$_4$]$^+$, 100), 479 ([MH]$^+$, 3), 461, 285 *m/z*
ESI-MS:	Instrument:	Perkin-Elmer Sciex API 300
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH$_4$OAc, 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:	523 (54), 501 (84), 496(57), 479 (2), 461 (10), 445 (6), 285 (100) *m/z*
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH$_4$OAc: MeOH (1:1)
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range m/z 100-600
	Major ions:	477 ([M]-, 100) *m/z*
HRMS:	Found:	501.246 *m/z*; C$_{24}$H$_{38}$O$_8$Na (MNaH$^+$)
	Requires:	501.246 *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm-1, KBr pellet
	Peaks:	3448, 1736, 1615, 1426, 1159, 1072, 1034 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	D$_2$O
	Key spectral data:	δ 0.91 (3H, s), 0.97 (3H, s), 4.48 (1H, d), 4.79 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	D$_2$O
	Spectral data:	δ 12.5, 14.2, 21.8, 21.9, 28.1, 30.1, 31.3, 34.5, 36.1, 36.2, 37.0, 42.8, 42.9, 49.1, 49.2, 51.6, 72.3, 73.4, 76.1, 76.3, 76.7, 100.8, 108.7, 152.6, 176.2, 229.5 ppm
Melting point:		224-226 °C (decomposition)
Microanalysis:	Found:	C = 51.1 %, H = 7.0 % (September 2008)
	Calculated:	C = 62.4 %, H = 7.5 % (Calculated for C$_{26}$H$_{37}$O$_8$Na)