Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

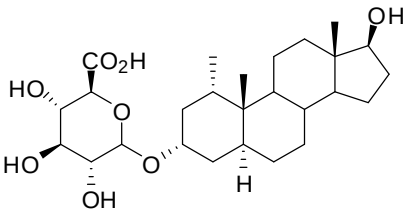
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D599: 1α-Methyl-5α-androstan-3α,17β-diol-3-β-D-glucuronic acid

Report ID: D599.2019.02 (Ampouled 110221)

Chemical Formula: $C_{26}H_{42}O_8$

Molecular Weight: 482.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-17	362499-11-4	879 µg

Synonyms: Mesterolone M2 3β-glucuronic acid

Expiration of certification: The property values are valid till 18 February 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. 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 reference material is intended for a single use to prepare a standard solution containing D599. 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.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. Acetonitrile then water). This will transfer 879 µg of anhydrous 1α-methyl-5α-androstan-3α,17β-diol-3-β-D-glucuronic acid. 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.
17 February 2020

This report supersedes any issued prior to 17 February 2020

Characterisation Report:

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	XBridge C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	A: 20 mM ammonium acetate buffer, pH=4.2, B: Acetonitrile Gradient: 0-8 min 30% B, 8-10 min 30-70% B, 10-13 min 70% B, 13-14 min 70-30% B, 14-30 min 30% B.
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	A: 20 mM ammonium acetate buffer, pH=4.2, B: Acetonitrile 0-13 min 30% B, 13-15 min 30-70% B, 15-20 min 70% B, 20-22 min 70-30% B, 22-30 min 30% B
	Flow Rate:	1.0 mL/min
	Column oven:	40 °C
	Detector:	Waters 2420 or 2424 ELS Detector
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.5%, s = 0.04% (7 ampoules in duplicate, November 2011)
	Re-analysis:	Mean = 99.5%, s = 0.02% (5 ampoules in duplicate, January 2013)
	Re-analysis:	Mean = 98.4%, s = 0.1% (5 ampoules in duplicate, March 2016)
	Re-analysis:	Mean = 99.0%, s = 0.1% (5 ampoules in duplicate, February 2019)

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, 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 Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/50 mM ammonium acetate, pH 4.2 [30:70]
	Flow Rate:	1.0 mL/min
	Detector:	Waters 2420 ELS Detector
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.04% (2 sub samples in duplicate, November 2004)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltech Alltima C-18 5 µm (4.6 mm x 150 mm)
	Column oven:	30 °C
	Mobile Phase:	A: 20 mM ammonium acetate buffer, pH=4.2, B: Acetonitrile 0-13 min 30% B, 13-15 min 30-70% B, 15-20 min 70% B, 20-22 min 70-30% B, 22-30 min 30% B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2420 ELS Detector
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.0%, s = 0.04% (5 sub samples in duplicate, November 2010)
Karl Fischer analysis:		Moisture content 11.3% mass fraction (November 2010)

Spectroscopic and other characterisation data

LC/MS:	Instrument:	Perkin-Elmer Sciex API 300
	Column:	Phenomenex LUNA C18 5 µm (1 mm x 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, postcolumn split 1:10
	The retention time of the material is reported along with the major peaks observed in the positive ion mass spectrum. The latter are reported in *m/z* and (in brackets) their assignment and percentage relative to the base peak.	
	13.2 min:	505 ([MNa]+, 27), 500 ([MNH4]+, 100), 483 ([MH]+, 3), 465, 289 *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 *m/z* 100-600
	Major ions:	505 (100), 500 (48), 483 (3), 465, 445, 289, 271 *m/z*
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH4OAc: MeOH (1:1)
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion,
	Scan range	100-600 *m/z*
	Major ions:	481 ([M-H]-, 100) *m/z*
HRMS:	Found:	483.294 *m/z*;
	Requires:	483.296 *m/z* $C_{26}H_{43}O_8$ $(MH)^+$
IR:	Instrument:	Perkin Elmer-IR
	Range:	4000-400 cm^{-1}, Nujol mull
	Peaks:	3415, 1727, 1641, 1448, 1380, 1257, 1089, 1024 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	CD_3OD (3.31 ppm)
	Key spectral data:	δ 0.75 (3H, s), 0.92 (3H, s), 1.08 (3H, d), 3.98 (1H, br s), 4.39 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker Advance-300
	Field strength:	75 MHz Solvent: CD_3OD (49.0 ppm)
	Spectral data:	δ 12.2, 15.0, 17.3, 21.4, 24.7, 30.3, 31.1, 33.1, 33.5, 34.2, 36.2, 37.2, 37.4, 38.4, 39.6, 44.6, 50.6, 53.1, 73.6, 75.5, 76.9, 77.1, 78.2, 83.0, 103.9, 173.1 ppm Signal due to C-17 (at > 220 pm) was not detected under the analysis conditions
Melting point:		158-159 °C
Microanalysis:	Found:	C = 57.8%; H = 9.0% (December, 2010)
	Calculated:	C = 56.3%; H = 9.1% (Calculated for $C_{26}H_{42}O_8.3H_2O$)
	Calculated:	C = 58.2%; H = 9.0% (Calculated for $C_{26}H_{42}O_8.4H_2O$)