Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

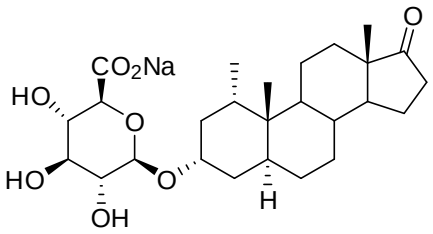
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D598: 1α-Methyl-5α-androstan-3α-ol-17-one-3-O-β-glucuronide, sodium salt

Report ID: D598.2023.01 (Ampouled 110228)

Chemical Formula: $C_{26}H_{39}NaO_8$

Molecular Weight: 502.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-S-16	Not available	913 ± 17 μg

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

Synonyms: Mesterolone metabolite 1 glucuronide (sodium salt).

Expiration of certification: The property values are valid till 12 December 2033, 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 sample bottles 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 D598. Off-white powder 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 assigned 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. a combination of methanol and water). This will transfer 913 ± 17 μg of anhydrous 1α-methyl-5α-androstan-3α-ol-17-one-3β-D-glucuronic acid, 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 three 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 ELSD detection on seven randomly selected 1-2 mg sub samples 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 a 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.
21 December 2023

This report supersedes any issued prior to 21 December 2023.

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:

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler OR Thermo Scientific Vanquish Flex UHPLC
	Column:	X-Bridge C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/10 mL of formic acid in 1 L of Milli-Q water, pH=2.2 (60:40)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2420 or 2424 ELS detector OR Vanquish Charged Aerosol Detector.
	Relative peak area response of main component:	
	Initial analysis:	Mean = 100.0%, s = 0.01% (7 ampoules in duplicate, October 2011)
	Re-analysis:	Mean = 100.0%, s = 0.05% (5 ampoules in duplicate, March 2012)
	Re-analysis:	Mean = 100.0%, s = 0.02% (5 ampoules in duplicate, September 2015)
	Re-analysis:	Mean = 100.0%, s = 0.01% (5 ampoules in duplicate, September 2018)
	Re-analysis:	Mean = 100.0%, s = 0.01% (5 ampoules in duplicate, June 2021)
	Re-analysis:	Mean = 100.0%, s = 0.01% (5 ampoules in duplicate, December 2023)

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 ELS detection, 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 thermogravimetric analysis and elemental microanalysis.

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 70% A for 13 mins, reduce A to 30% in 2 min, elute for 5 mins, increase A to 70% in 2 min, elute for 3 mins
	Flow rate:	1.0 mL/min
	Detector:	Waters 2420 ELS Detector
	Relative peak area of main component:	
	Initial analysis:	Mean = 100.0%, s = 0.0% (5 sub samples in duplicate, November 2010)
Thermogravimetric analysis:		Volatile content 8.3% and non volatile residue < 0.2% mass fraction (November 2010)
Karl Fischer analysis:		Moisture content 9.3% mass fraction (November 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)
	Mobile Phase:	A: 15 mM ammonium acetate, pH 4.2: methanol (9:1) B: Methanol: 15 mM ammonium acetate, pH 4.2 (9:1) 40% B to 90% B in 15 min
	Flow Rate:	100 μL/min, post column split 1:10
	The retention time is reported along 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 percentage relative to the base peak.	
	14.7 min:	503 ($[M+Na]^+$, 20), 498 ($[M+NH_4]^+$, 100), 481 ($[M+H]^+$, 3), 463, 287 *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:	525 (72), 503 (100), 498 (46), 481 (2), 461 (10), 463 (32), 445 (6), 287 (80) *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:	479 ($[M-H]^-$, 100) *m/z*
HRMS:	Found:	*m/z* 503.263; $C_{26}H_{40}O_8Na$ $(M+Na+H)^+$ requires m/z 503.262 *m/z*
IR:	Instrument:	Perkin Elmer-IR
	Range:	4000-400 cm^{-1}, Nujol mull
	Peaks:	3522, 3463, 1733, 1615, 1454, 1404, 1294, 1066, 1024 cm^{-1}
^1H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 0.88 (3H, s), 0.88 (3H, s), 0.98 (3H, d), 4.08 (1H, br s), 4.51 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker Gyro-300
	Field strength:	75 MHz
	Solvent:	D_2O
	Spectral data:	δ 13.7, 13.8, 16.1, 19.5, 21.8, 28.6, 30.7, 31.0, 31.3, 32.8, 34.7, 35.1, 35.1, 36.4, 38.0, 48.7, 49.1, 51.5, 72.4, 73.6, 75.5, 76.3, 76.5, 100.5, 176.3 ppm
Melting point:		224-226 °C
Microanalysis:	Found:	C = 57.0%; H = 8.1% (December 2010)
	Calculated:	C = 62.1%; H = 7.8% (Calculated for $C_{26}H_{39}NaO_8$)
	Calculated:	C = 56.1%; H = 8.2% (Calculated for $C_{26}H_{39}NaO_8.3H_2O$)