Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

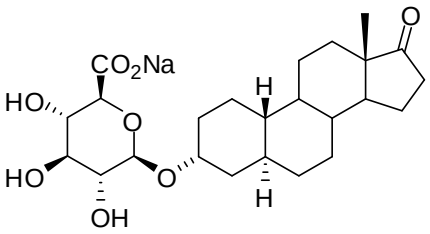
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D596a: 19-Norandrosterone glucuronic acid Na salt

Report ID: D596a.2021.02 (Ampouled 140821)

Chemical Formula: $C_{24}H_{35}O_8Na$

Molecular Weight: 474.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-14	Not Available	910 ± 21μg

IUPAC name: Sodium (3α,5α)-3-hydroxyestran-17-one β-D-glucopyranosiduronate.

Expiration of certification: The property values are valid till 7 September 2026, 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 D596a. 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 910 μg of anhydrous 19-norandrosterone glucuronic acid (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.
1 November 2022

This report supersedes any issued prior to 1 November 2022.

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 autosampler or Waters alliance 2695 separation module
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate (pH 4.2) (30:70 or gradient)
	Flow Rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean =100%, s = 0.0% (7 ampoules in duplicate, September 2014)
	Re-analysis	Mean =100%, s = 0.0% (5 ampoules in duplicate, November 2015)
	Re-analysis	Mean =100%, s = 0.0% (5 ampoules in duplicate, October 2018)
	Re-analysis	Mean =100%, s = 0.0% (5 ampoules in duplicate, September 2021)

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 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 elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate (pH 4.2) (30:70)
	Flow Rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.0% (3 sub samples in duplicate, March 1999)
	Re-analysis	Mean = 99.97%, s = 0.007% (5 sub samples in duplicate, March 2008)
	Re-analysis	Mean =100%, s = 0.0% (5 sub samples in duplicate, September 2014)
Karl Fischer analysis:		Moisture content 9.6% mass fraction (February to June 2005) Moisture content 9.6% mass fraction (March 2008) Moisture content 9.6% mass fraction (September 2014)

Spectroscopic and other characterisation data

GC-MS:	Persilylated derivative:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm
	Program:	200 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	280 °C
	Transfer line temp:	300 °C
	Carrier:	Helium
	Split ratio:	15/1
	The retention time of the persilylated derivative is reported along with the major peaks observed in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak. The molecular ion was not observed.	
	Per-TMS (11.3 min):	490 (16), 331 (48), 305 (22), 292 (24), 217 (100), 204 (61), 73 (93) *m/z*
ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI spray voltage at 4.5 kV negative ion
	Peak:	451.2 $(M\text{-}Na^+)^-$ *m/z*
FAB-MS:	Ions:	497 $(M\text{-}Na)^+$, 475 $(M\text{-}H)^+$, 413, 391, 329, 307, 289 *m/z*
	Ionisation:	15 kV in NBA/MeOH
HRMS:	Found:	475.2275 *m/z*; $C_{24}H_{36}O_8Na$ (MH^+)
	Requires:	475.2308 *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3448, 1736, 1614, 1408, 1296, 1163, 1068, 1037 cm^{-1}
^1H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.90 (3H, s), 3.22 (1H, dd, *J* = 1.2, 7.9 Hz), 3.41 (1H, t, *J* = 9.0 Hz), 3.44 (1H, dd, *J* = 8.9, 9.6 Hz), 3.57 (1H, d, *J* = 9.6 Hz), 4.12 (1H, br. s), 4.33 (1H, d, *J* = 7.8 Hz) ppm
^{13}C NMR:	Instrument:	Bruker DMX-600
	Field strength:	151 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 13.2, 21.6, 24.0, 25.0, 29.0, 30.0, 36.5, 39.4, 41.1, 47.3, 47.6, 51.1, 72.8, 72.9, 74.1, 75.3, 77.0, 101.1, 176.0 ppm
Melting point:		215-218 °C
Microanalysis:	Found:	C = 55.1%; H = 7.5% (August 2003)
	Calculated:	C = 55.0%; H = 7.8% (Calculated for $C_{24}H_{35}O_8Na$ + 9.4% water)