



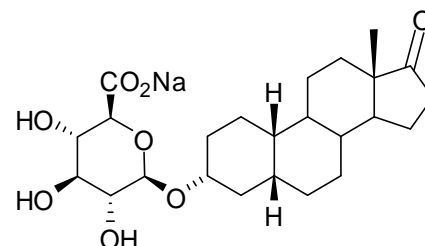
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D595a: 19-Noretiocholanolone glucuronic acid sodium salt

Report ID: D595a.2019.01 (Ampouled 100322)

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-13	Not available	816 µg

IUPAC name: Sodium (3 α ,5 β)-17-Oxoestrane-3-yl β -D-glucopyranosiduronate

Expiration of certification: The property values are valid till 25 June 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 RM is intended for a single use to prepare a standard solution containing D595a. Material was prepared by synthesis, 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 816 µg of anhydrous 19-noretiocholanolone glucuronide. 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 °C in a closed container in a dry, dark area.

Stability: This material has demonstrated stability over a minimum period of five 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.
13 February 2020

This report supersedes any issued prior to 13 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: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltima C-18, 5 μ m (4.6 mm x 150 mm)
Mobile Phase: A = 20 mM ammonium acetate buffer (pH 4.2); B = Acetonitrile
0-8 min 28% B; 8-9 min 28-52% B; 9-12 min 52% B
Flow Rate: 1.0 mL/min
Detector: Waters ELSD 2424
Relative peak area of the main component:
Initial analysis: Mean = 99.9%, s = 0.03% (7 ampoules in duplicate, July 2010)
Re-analysis: Mean = 100.0%, s = 0.0% (5 ampoules in duplicate, August 2011)
Re-analysis: Mean = 100.0%, s = 0.02% (5 ampoules in duplicate, July 2014)
Re-analysis: Mean = 100.0%, s = 0.0% (5 ampoules in duplicate, June 2019)

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 by qNMR was obtained using a combination of the three-proton singlet at 0.79 ppm and the one-proton multiplet at 2.9 ppm measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis, ^1H NMR spectroscopy, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

QNMR: Instrument: Bruker DMX-400
Field strength: 400 MHz
Solvent: DMSO- d_6
Internal standard: Dimethyl terephthalate (100% mass fraction)
Initial analysis: Mean = 81.0%, s = 0.7% (3 sub samples in duplicate, September 2011)

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltima C-18, 5 μ m (4.6 mm x 150 mm)
Mobile Phase: Acetonitrile/20 mM ammonium acetate (pH 4.2) [25:75]
Flow Rate: 1.0 mL/min
Detector: Waters ELSD 2424
Relative peak area of the main component:
Initial analysis: Mean = 99.9%, s = 0.02% (7 sub samples in duplicate, July 2010)

Thermogravimetric analysis: Volatiles content 12.1% mass fraction. Non-volatile residue was not determined (November 2007)

Karl Fischer analysis: Moisture content 12.3% mass fraction (November 2007)
Moisture content 12.2% mass fraction (March 2010)

Spectroscopic and other characterisation data

GC-MS:	<i>Persilylated</i> derivative: Instrument: HP6890/5973 Column: HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm Program: 200 °C, 10 °C/min to 300 °C (3 min) Injector: 280 °C Transfer line temp: 300 °C Carrier: Helium, 1.0 mL/min Split ratio: 15/1
	The retention time of the <i>persilylated</i> derivative is reported along with the major peaks 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 is not observed.
	<i>Per</i> -TMS (11.5 min): 490 (7), 331 (39), 305 (15), 292 (16), 217 (66), 204 (46), 73 (100) <i>m/z</i>
ESI-MS:	Instrument: Finnigan MAT TSQ 700 Operation: Negative ion mode, direct infusion Ionisation: ESI probe at 4.5 kV Peak: 451.2 <i>m/z</i> (M-Na) ⁻
FAB-MS:	Ions: 497(M-Na) ⁺ , 475 (MH) ⁺ , 439, 413, 329, 286 <i>m/z</i> Ionisation: 15 kV in NBA/MeOH
HRMS:	Found <i>m/z</i> 497.209; C ₂₄ H ₃₅ O ₈ Na ₂ (MNa ⁺) requires <i>m/z</i> 497.213 <i>m/z</i>
IR:	Instrument: Perkin-Elmer FT-IR Range: 4000-400 cm ⁻¹ , Nujol mull Peaks: 3488, 1733, 1609, 1454, 1376, 1159, 1078, 1026 cm ⁻¹
¹ H NMR:	Instrument: Bruker DMX-500 Field strength: 500 MHz Solvent: MeOH- <i>d</i> ₄ (3.31 ppm) Key spectral data: δ 0.91 (3H, s), 3.20 (1H, dd), 3.41 (1H, t), 3.44 (1H, dd), 3.58 (1H, d), 3.84 (1H, m), 4.45 (1H, d) ppm Ethanol was observed in the ¹ H NMR at 1.5% mass fraction (May 2000, December 2007 and September 2011)
¹³ C NMR:	Instrument: Bruker DMX-500 Field strength: 126 MHz Solvent: MeOH- <i>d</i> ₄ (49.0 ppm) Spectral data: δ 14.2, 22.6, 26.0, 26.2, 26.7, 26.9, 32.5, 32.9, 35.1, 36.8, 37.2, 40.0, 41.6, 42.6, 51.9, 73.8, 75.0, 76.2, 77.9, 79.3, 101.9, 177.0, 228 ppm One signal obscured by solvent peak
Microanalysis:	Found: C = 52.6%; H = 7.4% (September 2010) Calculated: C = 60.8%; H = 7.4% (Calculated for C ₂₄ H ₃₅ O ₈ Na)