Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

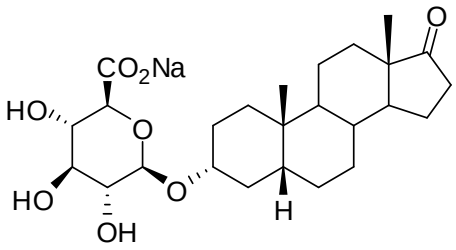
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D607: Etiocholanolone-3-*O*-β-glucuronide, sodium salt

Report ID: D607.2020.01 (Ampouled 090218)

Chemical Formula: $C_{25}H_{37}NaO_8$

Molecular Weight: 488.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-001742	3602-09-3 (free acid)	870 ± 45 μg

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

IUPAC name: (3α)-17-Oxo-5β-androstan-3-yl β-D-glucopyranosiduronic acid (Na salt)

Expiration of certification: The property values are valid till 6 July 2025, 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 sample bottles 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 certified reference material is intended for a single use to prepare a standard solution containing D607. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

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 870 ± 45 μg of anhydrous etiocholanolone 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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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 evaporative light scattering 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.
8 July 2020

This report supersedes any issued prior to 8 July 2020.

Characterisation Report:

HPLC:	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	20 mM NH_4OAc buffer, pH 4.2/Acetonitrile (A/B) Isocratic, 30% B
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean = 100%, s = 0.00% (7 ampoules in duplicate, February 2009)
	Re analysis:	Mean = 99.8%, s = 0.09% (5 ampoules in duplicate, June 2010)
HPLC:	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	20 mM NH_4OAc buffer, pH 4.2/Acetonitrile/ (A/B) Gradient 0-8 min 30% B, 8-12 min 30-80% B, 12-15 min 80% B, 15-20 min 80-30% B, 20-25 min 30% B
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean = 100%, s = 0.00% (5 ampoules in duplicate, June 2011)
HPLC:	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	20 mM NH_4OAc buffer, pH 4.2/Acetonitrile/ (A/B) Gradient 0-7 min 30% B, 7-8 min 30-80%B, 8-13 min 80% B, 13-14 min 80-30% B, 14-20 min 30% B
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.02% (5 ampoules in duplicate, May 2012)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, May 2015)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, July 2020)

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 quantitative nuclear magnetic resonance (qNMR) using the one proton multiplet at 3.85 ppm measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by HPLC with evaporative light scattering detection, Karl Fischer analysis, and ^1H NMR spectroscopy.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis:	Mean (3.85 ppm) = 87.5%, s = 0.6% (5 sub samples, November 2005)
	Re-analysis:	Mean (3.85 ppm) = 86.9%, s = 1.2% (3 sub samples, April 2009)
HPLC:	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate (pH 4.2) [30:70]
	Flow Rate:	1.0 mL/min
	Detector:	ELSD Retention time: 8.1 min
	Relative peak area of main component:	
	Initial analysis:	Mean = > 98% (3 sub samples)
Thermogravimetric analysis:		Volatile content 9.1% mass fraction (October 2005) Volatile content 8.8% mass fraction (January 2009) Non volatile residue was not determined
Karl Fischer analysis:		Moisture content 10.3% mass fraction (February 2009)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP6890 / 5973	
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 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 persilylated 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 was not observed.

Per-TMS (11.1 min): 504 (34), 375 (22), 345 (95), 217 (98), 204 (85), 73 (100) *m/z*

ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI probe at 4.5 kV
	Peak:	465.5 $(M\text{-}H^+)^-$ *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3617, 3450, 1731, 1615, 1410, 1166, 1072 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz Solvent: MeOH-d_4 (3.31 ppm)
	Key spectral data:	δ 0.86 (3H, s), 0.97 (3H, s), 3.81 (1H, m), 4.40 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz Solvent: MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 12.7, 19.7, 21.2, 22.3, 25.0, 26.0, 26.5, 31.5, 33.5, 34.5, 34.8, 35.2, 35.3, 40.6, 42.1, 51.3, 72.2, 73.5, 74.6, 76.4, 77.8, 100.3, 175.4, 222.6 ppm