



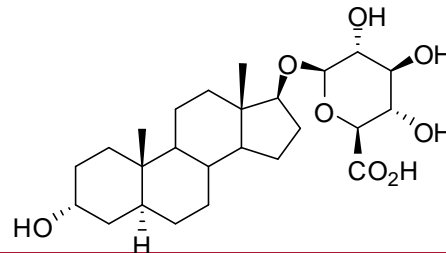
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S002: 5 α -Androstane-3 α , 17 β -diol-17-O- β -glucuronic acid

Report ID: S002.2017.03

Chemical Formula: C₂₅H₄₀O₈

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
09-S-06	95237-44-8	87.5 \pm 2.5 %

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

Synonyms: (3 α ,5 α ,17 β)-Hydroxyandrostan-17yl- β -D-glucopyranosiduronic acid
3 α -Hydroxy-5 α -androstan-17 β -yl-glucosiduronic acid
5 α -Androstane-3 α -ol-17 β -O-glucuronide

Expiration of certification: The property values are valid till 16 May 2020, i.e. three 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: Off-white powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

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

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 4 $^{\circ}$ C 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 water, volatiles and non-volatiles 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 three 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 ELS 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 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.
18 December 2019

This report supersedes any issued prior to 18 December 2019.

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.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see <http://www.bipm.org>).

Legal notice: Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA's behalf, assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this document.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with ELS detection thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

The purity value by qNMR was obtained using a combination of the one-proton doublet at 4.0 ppm and the one-proton doublet at 4.4 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Column:	Symmetry C-18, 5 μ m (4.6 mm \times 150 mm)
	Mobile Phase:	Methanol/Milli Q water (60:40) The aqueous phase was adjusted to pH 2.3 with formic acid
	Flow Rate:	1.0 mL/min
	Column oven:	40 $^{\circ}$ C
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.1% (7 sub samples in duplicate, June 2011)
	Re-analysis:	Mean = 99.6%, s = 0.03% (5 sub samples in duplicate, May 2012)
	Re-analysis:	Mean = 99.8%, s = 0.05% (5 sub samples in duplicate, May 2013)
	Re-analysis:	Mean = 99.9%, s = 0.02% (5 subsamples in duplicate, April 2014)
	Re-analysis:	Mean = 100%, s = 0.01% (5 subsamples in duplicate, May 2017)
Thermogravimetric analysis:	Volatile content 10.2% Non-volatile residue < 0.2% mass fraction (June 2011)	
Karl Fischer analysis:	Moisture content 11.5% mass fraction (June 2011) Moisture content 12.0% mass fraction (May 2012) Moisture content 11.6% mass fraction (May 2013) Moisture content 11.5% mass fraction (April 2014) Moisture content 12.1% mass fraction (April 2017)	
QNMR:	Instrument:	Bruker Avance-DMX 600
	Field strength:	600 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Internal standard:	Maleic acid (98.7% mass fraction)
	Initial analysis:	Mean (4.4 ppm) = 88.1%, s = 0.9% (6 sub samples, January 2011)
	Initial analysis:	Mean (4.0 ppm) = 88.1%, s = 1.0% (6 sub samples, January 2011)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 10 μ L/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	20 V
	Peak:	467.3 (M-H ⁺) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	No solvents detected.
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Chloroform/methanol (2/1) Single spot observed, R _f = 0.8. Visualisation with vanillin
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm ⁻¹ , KBr powder
	Peaks:	3406, 3319, 2914, 1728, 1448, 1354, 1253, 1165, 1059, 1031, 1003 cm ⁻¹
¹ H NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	600 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 0.76 (1H, m), 0.82 (3H, s), 0.83 (3H, s), 0.90-1.02 (2H, m), 1.14-1.70 (17H, m), 1.96-2.02 (2H, m), 3.20 (1H, dd, <i>J</i> = 7.9, 9.2 Hz), 3.35 (1H, t, <i>J</i> = 9.1 Hz), 3.51 (1H, t, <i>J</i> = 9.6 Hz), 3.67 (1H, t, <i>J</i> = 8.6 Hz), 3.73 (1H, d, <i>J</i> = 9.8 Hz), 3.95 (1H, m), 4.37 (1H, d, <i>J</i> = 7.8 Hz) ppm
¹³ C NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	150 MHz
	Solvent:	DMSO- <i>d</i> ₆ (39.5 ppm)
	Spectral data:	δ 11.1, 11.4, 19.9, 22.8, 28.2, 28.5, 28.6, 31.4, 32.0, 34.9, 35.7, 35.8, 36.9, 38.6, 42.7, 50.4, 54.1, 64.1, 71.5, 73.4, 75.7, 76.1, 87.7, 103.5, 170.4 ppm
Melting point:		256-257 $^{\circ}$ C
Microanalysis:	Found:	C = 57.0 %; H = 9.0 % (June 2011)
	Calculated:	C = 64.1 %; H = 8.6 % (Calculated for C ₂₅ H ₄₀ O ₈)
	Calculated:	C = 57.1 %; H = 8.9 % (Calculated for C ₂₅ H ₄₀ O ₈ + 11.0% H ₂ O)