Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

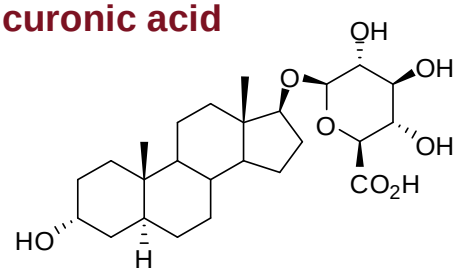
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

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

Report ID: S002.2025.01 (Ampouled 240109)

Chemical Formula: $C_{25}H_{40}O_8$

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
09-S-06	95237-44-8	874 ± 24 µg

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

IUPAC name: (3β,5β,8α,9β,14β,17α)-3-Hydroxyandrostan-17-yl β-D-glucopyranosiduronic acid

Expiration of certification: The property values are valid till 4 February 2028, 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 ampoules 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. This CRM is intended for a single use to prepare a standard solution of 5α-androstan-3α,17β-diol-17-*O*-β-glucuronic acid. This material was prepared by synthesis and certified for identity and purity by NMI Australia.

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 874 ± 24 µg of anhydrous 5α-androstan-3α,17β-diol-17-*O*-β-glucuronic acid. 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 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: In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years. The measurement uncertainty at the 95% confidence interval also includes a stability component determined from accelerated stability trials conducted at 40 ºC and 75% humidity for 14 days.

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 UHPLC with Charged Aerosol 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 2025

This report supersedes any issued prior to 13 February 2025.

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:

UHPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Symmetry C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v) with 0.1% formic acid in both the methanol and MilliQ water. The aqueous phase was adjusted to pH 2.3 with formic acid.
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.1%, s = 0.08% (7 ampoules in duplicate, February 2024)
	Re analysis:	Mean = 97.5%, s = 0.24% (5 ampoules in duplicate, February 2025)
HPLC:	Column:	Symmetry C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v) with 0.1% formic acid in both the methanol and MilliQ water.
	Flow Rate:	1.0 mL/min
	Column oven:	40 °C
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.03% (5 ampoules in duplicate, February 2025)

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 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 ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) 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 μm (4.6 mm × 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 °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 = 100.0%, s= 0.01% (5 subsamples in duplicate, May 2017)
	Re-analysis:	Mean = 100.0%, s= 0.01% (5 subsamples in duplicate, April 2020)
Thermogravimetric analysis:		Volatile content 10.2% Non-volatile residue < 0.2% mass fraction (June 2011)
Karl Fischer analysis:		Moisture content 11.7 ± 0.6% (June 2011 – May 2020)
QNMR:	Instrument:	Bruker Avance-DMX 600
	Field strength:	600 MHz
	Solvent:	MeOH-d_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 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	20 V
	Peak:	467.3 ($M-H^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °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-d_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, *J* = 7.9, 9.2 Hz), 3.35 (1H, t, *J* = 9.1 Hz), 3.51 (1H, t, *J* = 9.6 Hz), 3.67 (1H, t, *J* = 8.6 Hz), 3.73 (1H, d, *J* = 9.8 Hz), 3.95 (1H, m), 4.37 (1H, d, *J* = 7.8 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	150 MHz
	Solvent:	DMSO-d_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 °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_2O)