Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

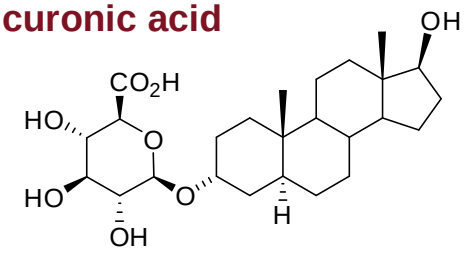
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S004b: 5α-Androstane-3α, 17β-diol-3-O-β-glucuronic acid

Report ID: S004b.2024.01

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

Molecular Weight: 468.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
24-S-01	65535-18-4	82.7 ± 1.2%

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

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

Expiration of certification: The property values are valid till 11 October 2027, three years from the date of 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. 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: Off-white powder prepared by synthesis and certified for identity and purity by NMI Australia. Packaged in amber glass bottles with a septum and crimped aluminium 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 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 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 charged aerosol detection on ten 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 a 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.
16 December 2024

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:

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 evaporative light scattering and charged aerosol 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.

Supporting evidence is provided by quantitative NMR (qNMR), and elemental microanalysis. The purity value obtained by qNMR was determined using combination of the protons at region 3.3-4.6 ppm against a certified internal standard of maleic acid.

HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	ACE Super 18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/0.1 percent formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	CAD Detector
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 95.2%, s = 0.09% (10 sub samples in duplicate, October 2024)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE Super 18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/0.1 percent formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.03% (10 sub samples in duplicate, October 2024)
Karl Fischer analysis:		Moisture content 13.2% mass fraction (October 2024)
Thermogravimetric analysis:		Non-volatile residue 2.3% mass fraction (October 2024)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	400 MHz
	Solvent:	AcOH-d_4 (2.03 ppm)
	Internal standard:	Maleic acid (99.9% mass fraction)
	Initial analysis:	Mean (3.3-4.6 ppm) = 82.3%, s = 0.1% (5 sub samples, October 2024)
	Initial analysis:	Mean 4.5 ppm) = 82.7%, s = 0.1% (5 sub samples, October 2024)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Shimadzu LC-TQ-MS 8045
	Operation:	Direct infusion at 10 μL/min
	Ionisation mode:	Electrospray negative ion
	Interface voltage:	3 kV
	Peak:	467.4 ($M-H^-$) *m/z*
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3423, 2930, 1717, 1446, 1362, 1261, 1162, 1066, 1035 cm^{-1}
^1H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.78 (1H, dt, *J* = 0.4, 12.5 Hz), 0.83 (3H, s), 0.87-1.05 (3H, m), 1.13-1.68 (15H, m), 1.80-1.85 (2H, m), 1.96 (1H, m), 3.24 (1H, dd, *J* = 8.0, 9.0 Hz), 3.38 (1H, t, *J* = 9.0 Hz), 3.53 (1H, t, *J* = 8.5 Hz), 3.55 (1H, t, *J* = 8.0 Hz), 3.76 (1H, d, *J* = 9.5 Hz), 3.94 (1H, m), 4.37 (1H, d, *J* =7.5 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 11.7, 11.9, 21.5, 24.3, 26.5, 29.5, 30.6, 32.8, 33.7, 35.3, 36.9, 37.0, 38.1, 40.5, 44.1, 52.5, 55.8, 73.2, 74.8, 75.7, 76.6, 77.6, 82.6, 103.0, 172.6 ppm
Microanalysis:	Found:	C = 54.6%; H = 8.8% (October 2024)
	Calculated:	C = 64.1%; H = 8.6% (Calculated for $C_{25}H_{40}O_8$)
	Calculated:	C = 54.3%; H = 8.7% (Calculated for $C_{25}H_{40}O_8$ + 4 H_2O + ¼ NaCl)