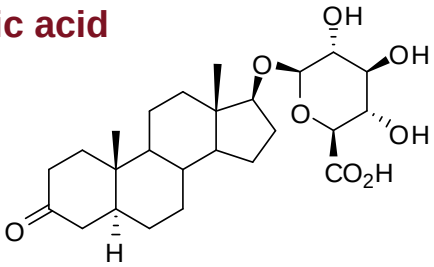
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S006: 5α-Dihydrotestosterone-O-β-D-glucuronic acid



Report ID: S006.2021.03

Chemical Formula: $C_{25}H_{38}O_8$

Molecular Weight: 466.6 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
10-S-02	**42037-24-1**	**93.5 ± 1.0%**

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

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

Expiration of certification: The property values are valid till 26 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White solid 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 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% confidence interval includes a stability component which has been estimated from annual 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 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.
15 November 2022

This report supersedes any issued prior to 15 November 2022.

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 quantitative nuclear magnetic resonance (qNMR) using the six protons between 3.4-4.6 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by mass balance using a combination of traditional analytical techniques including HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis, ^{1}H NMR spectroscopy, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

Note: This material has shown signs of decomposition in methanol.

QNMR:	Instrument:	Bruker Avance-500
	Field strength:	500 MHz
	Solvent:	Acetic acid-d_4 (2.03 ppm)
	Internal standard:	Maleic acid (98.7% mass fraction)
	Initial analysis:	Mean = 93.5%, s = 0.2% (3 sub samples in duplicate, June 2016)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Formic acid, pH = 2.3; B = Acetonitrile
		0-5 min 35% B, 6-13 min 70% B, 20 min 35% B
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.1% (5 sub samples in duplicate, April 2013)
	Re-analysis:	Mean = 98.9%, s = 0.06% (5 sub samples in duplicate, April 2016)
	Re-analysis:	Mean = 98.9%, s = 0.09% (5 sub samples in duplicate, June 2019)
Karl Fischer analysis:		Moisture content 6.9% mass fraction (May 2010) Moisture content 3.1% mass fraction (May 2011) Moisture content 3.5% mass fraction (May 2012) Moisture content 4.8% mass fraction (March 2013) Moisture content 3.9% mass fraction (February 2016) Moisture content 4.0% mass fraction (May 2019) Moisture content 4.0% mass fraction (March 2021)
Thermogravimetric analysis:		Volatile content 2.9% and non volatile residue content 1% mass fraction (July 2010)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	465.4 ($M\text{-}H^+$) *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:	Benzene and toluene
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (2/1) Single spot observed, R_f = 0.4. Visualisation with vanillin
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-500 cm^{-1}, KBr powder
	Peaks:	3379, 2932, 2846, 1717, 1443, 1409, 1363, 1253, 1174, 1060, 682 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.70 (1H, ddd, *J* = 4.0, 12.4, 12.4 Hz), 0.75 (3H, s), 0.78-0.98 (2H, m), 0.97 (3H, s), 1.05-1.54 (11H, m), 1.61 (1H, m), 1.81-1.95 (4H, m), 2.08 (1H, m), 2.29 (1H, t, *J* = 7.1 Hz), 2.41 (1H, m), 2.95 (1H, t, *J* = 8.5 Hz), 3.13 (1H, t, *J* = 9.0 Hz), 3.27 (1H, t, *J* = 9.5 Hz), 3.52 (1H, d, *J* = 9.8 Hz), 3.56 (1H, t, *J* = 8.6 Hz), 4.22 (1H, d, *J* = 7.8 Hz) ppm Benzene and toluene were not observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	125 MHz
	Solvent:	DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 11.1, 11.4, 20.5, 22.9, 28.3, 28.5, 30.8, 34.7, 35.3, 36.8, 37.6, 37.9, 42.7, 44.2, 46.0, 50.1, 53.1, 71.5, 73.4, 75.6, 76.1, 87.7, 103.5, 170.5, 210.5 ppm
Melting point:		NA
Microanalysis:	Found:	C = 60.5%; H = 8.1% (May 2010)
	Found:	C = 62.1%; H = 8.2% (November 2011)
	Calculated:	C = 64.4%; H = 8.2% (Calculated for $C_{25}H_{38}O_8$)
	Calculated:	C = 62.4%; H = 8.3% (Calculated for $C_{25}H_{38}O_8$ + 3.1% H_2O)
	Calculated:	C = 60.1%; H = 8.4% (Calculated for $C_{25}H_{38}O_8$ + 6.6% H_2O)