



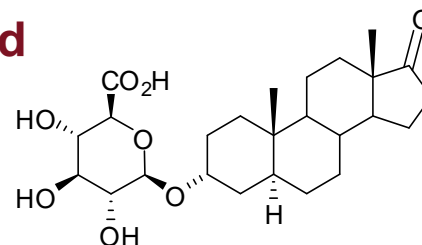
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D572b: Androsterone glucuronic acid

Report ID: D572b.2023.01 (Ampouled 190117)

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

Molecular Weight: 466.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
09-S-11	1852-43-3	885 ± 16 µg

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

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

Expiration of certification: The property values are valid till 4 July 2028, 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 ampoules 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 CRM is intended for a single use to prepare a standard solution containing D572b. 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 such as acetonitrile. This will transfer 885 ± 16 µg of anhydrous androsterone glucuronide. 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 °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 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 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.
10 July 2023

This report supersedes any issued prior to 10 July 2023.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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

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:

uHPLC:	Instrument:	Thermo Scientific Dionex Ultimate 3000 RS binary pump
	Column:	Phenyl-hexyl 150 mm x 4.6 mm x 3.0 µm particle size
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/0.1% Formic acid in MilliQ water (32:68 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Corona Ultra RS Charged aerosol detector (CAD)
	CAD temperature:	35 °C
	CAD nitrogen pressure:	35 psi
	Power function:	1.0
	Acquisition rate:	10 Hz
	Filter constant:	3.0
	Relative peak area of the main component:	
	Initial analysis:	Mean = 97.4%, s = 0.07% (7 ampoules in duplicate, February 2019)
	Re-analysis:	Mean = 97.5%, s = 0.22% (5 ampoules in duplicate, April 2020)
	Re-analysis:	Mean = 97.4%, s = 0.25% (5 ampoules in duplicate, April 2021)
	Re-analysis:	Mean = 97.2%, s = 0.04% (5 ampoules in duplicate, July 2023)

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). The one-proton doublet at 4.52 ppm was measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by HPLC-ELSD, Karl-Fischer, ¹H NMR spectroscopy and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	AcOH- <i>d</i> ₄ (2.03 ppm)
	Internal standard:	Potassium hydrogen maleate (99.7% mass fraction)
	Initial analysis:	Mean = 87.2%, s = 0.09% (5 sub samples, January 2019)
HPLC:	Instrument:	Shimadzu Model LC-20AB, SIL-20A HT auto sampler
	Column:	Alltima C18, 5 µm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Water plus formic acid, pH 2.3/methanol (35:65)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.07% (10 sub samples in duplicate, October 2010)
	Re-analysis:	Mean = 99.2%, s = 0.02% (1 sub samples in replicate, June 2012)
Thermogravimetric analysis:	Non-volatile residue < 0.2% mass fraction (November 2010)	
Karl Fischer analysis:	Moisture content 10.5% mass fraction (December 2010 and April 2012)	
	Moisture content 10.8% mass fraction (January 2019)	

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 5 $\mu\text{L}/\text{min}$
	Ionisation:	ESI spray voltage at 3.0 kV negative ion.
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	465.3 (M-H ⁺) <i>m/z</i>
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1} , neat
	Peaks:	3482, 2929, 2856, 1734, 1718, 1456, 1446, 1363, 1256, 1088, 1060, 1021 cm^{-1}
¹ H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 0.86 (3H, s), 0.87 (3H, s), 0.82-0.89 (1H, m), 1.00-1.10 (1H, m), 1.17-1.87 (17H, m), 1.91-1.98 (1H, m), 2.06 (1H, m), 2.43 (1H, dd, <i>J</i> = 10.6, 19.0 Hz), 3.24 (1H, dd, <i>J</i> = 7.9, 9.1 Hz), 3.38 (1H, t, <i>J</i> = 9.1 Hz), 3.53 (1H, t, <i>J</i> = 8.0 Hz), 3.77 (1H, d, <i>J</i> = 9.7 Hz), 3.95 (1H, m), 4.37 (1H, d, <i>J</i> = 7.8 Hz) ppm
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49 ppm)
	Spectral data:	δ 11.9, 14.2, 21.2, 22.7, 26.5, 29.3, 32.1, 32.9, 33.6, 35.3, 36.4, 36.7, 37.0, 40.4, 52.9, 55.7, 73.2, 74.8, 75.7, 76.6, 77.6, 103.1, 172.6, 224.2 ppm
Microanalysis:	Found:	C = 57.9%; H = 8.5% (December 2010)
	Calculated:	C = 57.7%; H = 8.5% (Calculated for C ₂₅ H ₃₈ O ₈ ·3H ₂ O)