



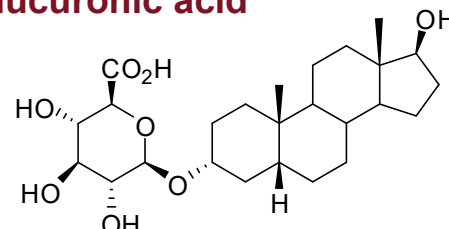
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S003c: 5 β -Androstane-3 α , 17 β -diol-3-O- β -glucuronic acid

Report ID: S003c.2024.01 (Ampouled 240606)

Chemical Formula: C₂₅H₄₀O₈

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
23-S-07	114761-91-0	949 ± 27 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 β ,17 β)-17-Hydroxyandrostane-3-yl β -D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 5 July 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 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. The CRM is intended for a single use to prepare a standard solution containing S003c. 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 (e.g. methanol). This will transfer 949 ± 27 μg of anhydrous 5 β -Androstane-3 α , 17 β -diol-3-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 °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 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 °C 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 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.
14 August 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:

UHPLC:	Instrument:	Thermo Scientific Vanquish pump, Vanquish Autosampler Vanquish column compartment.
	Column:	ACE Excel Super C-18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Methanol/ MilliQ water (60:40) Formic acid (0.2% v/v) was present in the aqueous phase
	Flow rate:	1.0 mL/min
	Detector:	Vanquish detector
Relative mass fraction of the main component:		
Initial analysis:	Mean = 99.36%, s = 0.01% (6 ampoules in duplicate, July 2024)	
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE, super C-18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Methanol/ MilliQ water (60:40) Formic acid (0.2% v/v) was present in the aqueous phase
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
Relative mass fraction of the main component:		
Initial analysis:	Mean = 99.9%, s = 0.02% (3 ampoules in duplicate, July 2024)	

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 mass balance from a combination of traditional analytical techniques, including HPLC with charged aerosol detection, thermogravimetric analysis, Karl Fischer analysis, and 1 H 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.

Supporting evidence is provided by quantitative NMR (qNMR), qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

The certified purity value by qNMR was obtained using the doublets at 4.6 ppm measured against a certified internal standard of potassium hydrogen maleate.

UHPLC:	Instrument:	Thermo Fisher Ultimate 3000RS Pump, RS autosampler, RS column compartment
	Column:	ACE SuperC18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Methanol/MilliQ water (60:40) Formic acid (0.2% v/v) was present in the aqueous phase
	Flow rate:	1.0 mL/min
	Detector:	Thermo Corona Ultra RS CAD Detector
Relative mass fraction of the main component:		
Initial analysis:	Mean = 99.5%, s = 0.03% (7 sub samples in duplicate, April 2024)	

Karl Fischer analysis: Moisture content 4.6% mass fraction (May 2024)

Thermogravimetric analysis: Volatiles content 4.8% and non-volatile residue 0.6% mass fraction (April 2024)

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	AcOH- d_4 (2.07 ppm)
	Internal standard:	Maleic acid (99.9% mass fraction)
	Initial analysis:	Mean (4.6 ppm) = 94.5%, s = 0.2% (5 sub samples, May 2024)

Spectroscopic and other characterisation data

GC-MS:	The free steroid was liberated upon treatment with β -glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA.
Instrument:	Agilent 6890/5973
Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μ m
Program:	180 $^{\circ}$ C (1 min), 30 $^{\circ}$ C/min to 250 $^{\circ}$ C (10 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
Injector:	250 $^{\circ}$ C
Transfer line temp:	280 $^{\circ}$ C
Carrier:	Helium
Split ratio:	20/1
	The retention time of the <i>bis</i> -TMS derivative of 5 β -androstane-3 α , 17 β -diol is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.
<i>Bis</i> -TMS (8.8min):	436 (M ⁺ , 4), 421 (8), 346 (27), 256 (79), 241 (73), 215 (49), 201 (29), 199 (27), 161 (119), 147 (23), 129 (89), 107 (25), 93 (27), 81 (26), 73 (100) <i>m/z</i>
TLC:	Conditions: Kieselgel 60F254. Chloroform/methanol (2/1) Single spot observed, R _f = 0.3. Visualisation with vanillin.
IR:	Instrument: Bruker Alpha FT-IR Range: 4000-400 cm ⁻¹ , neat Peaks: 3446, 3344, 2939, 2896, 1719, 1457, 1449, 1375, 1259, 1213, 1161, 1122, 1042, 999, 696 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 0.72 (3H, s), 0.96 (3H, s), 0.97-1.18 (4H, m), 1.19-1.52 (10H, m), 1.54-1.64 (2H, m), 1.76-2.03 (5H, m), 1.98 (1H, m), 3.19 (1H, dd, <i>J</i> = 8.1, 9.0 Hz), 3.38 (1H, t, <i>J</i> = 9.2 Hz), 3.52 (1H, t, <i>J</i> = 9.5 Hz), 3.57 (1H, t, <i>J</i> = 8.6 Hz), 3.69 (1H, m), 3.78 (1H, d, <i>J</i> = 9.7 Hz), 4.45 (1H, d, <i>J</i> = 7.7 Hz) ppm Two related impurities estimated at 0.5% and 0.1% mass fraction was observed in the ¹ H NMR (AcOH- <i>d</i> ₄) (May 2022)
¹³ C NMR:	Instrument: Bruker Avance III 500 Field strength: 126 MHz Solvent: MeOH- <i>d</i> ₄ (49.0 ppm) Spectral data: δ 11.6, 21.5, 23.9, 24.3, 27.2, 27.7, 28.2, 30.7, 35.3, 35.9, 36.3, 37.3, 38.2, 42.0, 43.6, 44.2, 52.4, 73.2, 74.8, 76.6, 77.5, 80.3, 82.6, 102.6, 172.7 ppm
Melting point:	210-211 $^{\circ}$ C
Microanalysis:	Found: C = 61.5%; H = 8.8% (April 2016) Calculated: C = 61.3%; H = 8.7% (Calculated for C ₂₅ H ₄₀ O ₈ with 4.3% water)