Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

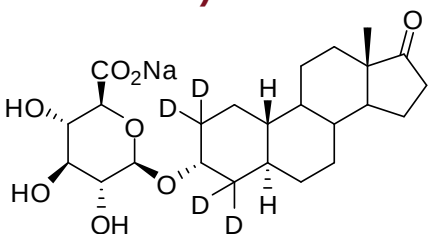
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D898b: d4-Norandrosterone-β-glucuronide (sodium salt)

Report ID: D898b.2022.01 (Ampouled 200213)

Chemical Formula: $C_{24}H_{31}D_4O_8Na$

Molecular Weight: 478.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
18-S-05	**Not available**	**892 μg**

IUPAC name: 2,2,4,4-d4-(3α,5α)-17-Oxoestran-3yl β-D-glucopyranosiduronic acid sodium salt.

Expiration of certification: The property values are valid till 15 July 2027, 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 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 deuterated internal standard is intended for a single use to prepare a standard solution containing D898b. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d_4-norandrosterone-β-glucuronide sodium salt. d_3-, d_2-, d_1- and d_0- Norandrosterone-β-glucuronide sodium salt are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_4, d_3, d_2 and d_1) and d_0- norandrosterone-β-glucuronide sodium salt in the material.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile : Milli-Q water (1:1)). This will transfer approximately 892 μg of anhydrous norandrosterone-β-glucuronide sodium salt (d_4, d_3, d_2, d_1 and d_0). 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.

Stability: 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 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 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.
11 August 2022.

This report supersedes any issued prior to 11 August 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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water; B = Acetonitrile 0-1 min 25% B; 1-2 min 25-30% B; 2-15 min 30%B; 15-16 min 30-50%B;16-17 min 50%B, 17-18 min 50-25%B, 18-30 min 25%B. The aqueous phase was buffered at pH 4.2 using 20mM NH_4OAc and AcOH
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.07% (7 ampoules in duplicate, February 2020)
	Re-analysis:	Mean = 99.7%, s = 0.03% (5 sub samples in duplicate, July 2022)

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 purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^1H 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 qualitative elemental microanalysis.

The main component of this material is d_4-norandrosterone-β-glucuronide sodium salt. d_3-, d_2-, d_1- and d_0-Norandrosterone-β-glucuronide sodium salt are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_4, d_3, d_2 and d_1) and d_0-norandrosterone-β-glucuronide sodium salt in the material.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity:	d_4 ≈ 81% [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.3% [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water; B = Acetonitrile 0-1 min 25% B, 1-2 min 25-30% B; 2-15 min 30% B; 15-16 min 30-50% B; 16-17 min 50% B, 17-18 min 50-25% B, 18-30 min 25% B. The aqueous phase was buffered at pH 4.2 using 20mM NH_4OAc and AcOH
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 100%, s = 0.01% (10 sub samples in duplicate, October 2018)
Karl Fischer analysis:		Moisture content 10.7 % mass fraction (November 2018)
Thermogravimetric analysis:		Volatile content 8.0% mass fraction (November 2018). The non volatile content could not be determined by thermogravimetric analysis as this material is a sodium salt.

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:	DB-5MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	180 °C (1 min), 30 °C /min to 250 °C (10 min), 30 °C /min to 300 °C (10 min)
Injector:	250 °C
Split ratio:	20/1
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Scan range:	50-700 *m/z*

The retention time of the *bis*-TMS derivative of d_4-norandrosterone 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.

Bis-TMS (8.2 min): 424 (M^+, 65), 409 (100), 319 (22), 229 (12), 182 (11), 169 (25), 73 (69) *m/z*

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3384, 2909, 2857, 1734, 1599, 1407, 1042, 1010 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III-500
Field strength:	500 MHz
Solvent:	MeOH-d_4 (3.31 ppm)
Spectral data:	δ 0.73-0.83 (2H, m), 0.89 (3H, s), 0.99-1.38 (7H, m), 1.51-1.66 (4H, m), 1.72-1.82 (2H, m), 1.87-1.91 (1H, m), 1.92-1.98 (1H, m), 2.07 (1H, ddd, *J* = 9, 9, 19 Hz), 2.43 (H, dd, *J* = 8.7 19.3 Hz), 3.22 (1H, dd, *J* = 8.1, 9.2 Hz), 3.40 (1H, t, *J* = 9.0 Hz), 3.46 (1H, t, *J* = 9.5 Hz), 3.57 (1H, d, *J* = 9.6 Hz), 4.09 (1H, s), 4.34 (1H, d, *J* = 7.8 Hz) ppm

In the ^{1}H NMR spectrum methanol was detected at 0.01% mass fraction.

^{13}C NMR:

Instrument:	Bruker Avance III-500
Field strength:	126 MHz
Solvent:	MeOH-d_4 (49 ppm)
Spectral data:	δ 14.2, 22.6, 24.8, 26.0, 31.1, 32.8, 34.6, 36.7, 37.3, 42.1, 48.1, 49.3, 49.6, 52.1, 73.8, 73.9, 75.0, 76.4, 77.9, 102.1, 177.2, 224.4 ppm

Melting point: 215-222 °C (Decomposition)

Microanalysis:

Found:	C = 55.7%; H = 8.1% (November 2018)
Calculated:	C = 53.8%; H = 7.9% (Calculated for $C_{24}H_{31}D_4O_8$ with 10.7% water)
Calculated:	C = 55.4%; H = 7.8% (Calculated for $C_{24}H_{31}D_4O_8$ with 8% water)