Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

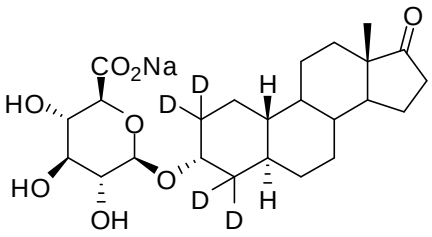
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

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

Report ID: D898b.2018.01

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

Molecular Weight: 478.5 g/mol



Certified value

Batch No.	CAS No.	Purity estimate
18-S-05	N/A	89.3 %

IUPAC name: 2,2,4,4-d_4-(3α,5α)-17-Oxoestran-3yl β-D-glucopyranosiduronic acid sodium salt.

Expiration of certification: The property values are valid till 24 October 2021, i.e. 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: White powder 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. 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.

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: 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.

Stability: This material has demonstrated stability over a minimum period of three 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-ELSD on ten randomly selected 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.
8 May 2019.

NATA logo notice: Accredited for compliance with ISO Guide 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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 ELS 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 elemental microanalysis.

The main component of this material is d_4-norandrosterone-β-glucuronide. d_3-, d_2-, d_1- and d_0-Norandrosterone-β-glucuronide 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 in the material.

Isotopic Purity: $d_4 \approx 81\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

$d_0 < 0.3\%$ [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

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 = MilliQ 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 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)
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.
Karl Fischer analysis:		Moisture content 10.7 % mass fraction (November 2018)

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 ºC	Transfer line temp: 280 ºC
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

The retention time of the *bis*-TMS derivative of d_4-19-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*$_4$ (49.0 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)