Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
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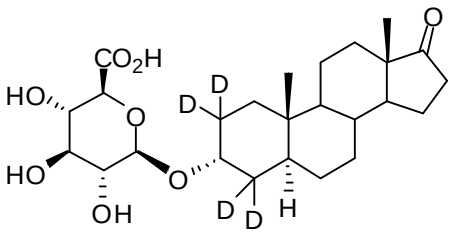
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D829f: d4-Androsterone-β-glucuronic acid

Report ID: D829f.2019.02 (Ampouled 190822)

Chemical Formula: $C_{25}H_{34}D_4O_8$

Molecular Weight: 470.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
14-S-10	**Not available**	**943 μg**

IUPAC name: d_4-(3α,5α)-17-Oxoandrostan-3-yl-β-D-glucopyranosiduronic acid

Expiration of certification: The property values are valid till 22 November 2022, 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 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 and is intended for a single use to prepare a standard solution containing D829f. The material was prepared by synthesisand certified for identity and purity by NMIA.

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. methanol). This will transfer approximately 943 μg of anhydrous androsterone-β-glucuronic acid (d_4, d_3, d_2, d_1 and d_0).

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.
6 March 2020.

This report supersedes any issued prior to 6 March, 2020.

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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	55 °C
	Mobile Phase:	Methanol/MilliQ water (60:40) The aqueous phase was buffered using 1% formic acid
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.06% (7 ampoules in duplicate, November 2019)

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 ^{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 qualitative elemental microanalysis.

The main component of this material is d_4-androsterone-β- glucuronic acid. d_3-, d_2-, d_1- and d_0-Androsterone-β- glucuronic acid 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-androsterone-β- glucuronic acid 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 ≈ 87% [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.2% [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	55 °C
	Mobile Phase:	Methanol/MilliQ water (65:35) The aqueous phase was buffered using 1% formic acid
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (10 sub samples in duplicate, November 2014)
	Re-analysis:	Mean = 99.9%, s = 0.03% (7 sub samples in duplicate, August 2018)
Karl Fischer analysis:		Moisture content 9.2% mass fraction (November 2014) Moisture content 5.4% mass fraction (June 2018)
Thermogravimetric analysis:		Volatile content 8.5% and non-volatile residue 0.4 % mass fraction (November 2014).

Spectroscopic and other characterisation data

GC-MS:

Bis-TMS derivative:
The free steroid was liberated upon treatment with β-glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA.

Instrument:	Shimadzu GCMS-QP2010 Plus
Column:	HP Ultra 1
Program:	185 °C (0.2 min), 3 °C/min to 236 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C (2 min)
Injector:	250 °C
Split ratio:	15/1
Transfer line temp:	230 °C
Carrier:	Helium
Scan range:	50-550 *m/z*

The retention time of the *bis*-TMS derivative of d_4-androsterone 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 (9.3 min): 438 (M^+, 26), 423 (42), 333 (23), 243 (15), 182 (12), 169 (23), 73 (100) *m/z*

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:	35 V
Peak:	469.4 $(M\text{-}H^+)^-$ *m/z*

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder.
Peaks:	3396, 2919, 2857, 2361, 2342, 2197, 2106, 1734, 1088, 1054, 1017 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III-500
Field strength:	500 MHz
Solvent:	MeOH-d_4 (3.31 ppm)
Spectral data:	δ 0.85 (3H, s), 0.87 (3H, s), 1.01-1.09 (1H, m), 1.18-1.44 (7H, m), 1.50-1.82 (6H, m), 1.92-1.97 (H, m), 2.06 (1H, m), 2.43 (1H, dd, *J* = 8.7, 19.2 Hz), 3.24 (1H, dd, *J* = 8.0, 9.2 Hz), 3.37 (1H, t, *J* = 9.1 Hz), 3.53 (1H, t, *J* = 9.4 Hz), 3.76 (1H, d, *J* = 9.8 Hz), 3.94 (1H, s), 4.37 (1H, d, *J* = 7.8 Hz) ppm

^{13}C NMR:

Instrument:	Bruker Avance III-400
Field strength:	126 MHz
Solvent:	MeOH-d_4 (49 ppm)
Spectral data:	δ 11.9, 14.2, 21.2, 22.7, 29.3, 32.1, 32.9, 33.5, 36.4, 36.7, 37.0, 40.3, 52.9, 55.7, 73.2, 74.8, 75.5, 76.6, 77.6, 103.0, 172.7, 224.2 ppm

Melting point: 149-152 °C

Microanalysis:

Found:	C = 60.4%; H = 8.3% (December 2015)
Calculated:	C = 60.4%; H = 8.3% (Calculated for $C_{25}H_{34}D_4O_8$ + 5.4% water)