Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

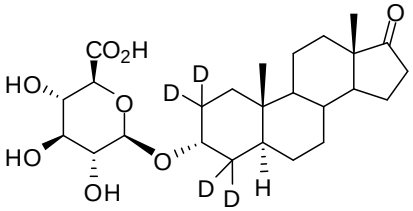
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D829g: d4-Androsterone-β-glucuronic acid

Report ID: D829g.2022.02 (Ampouled 220217)

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

Molecular Weight: 470.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
19-S-01	Not available	882 ± 23 μg

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

IUPAC name: (3α,5α)-17-Oxo(2,2,4,4-$^2H_4$)androstan-3-yl β-D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 17 March 2025, 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 under an atmosphere of argon. The deuterated internal standard is intended for a single use to prepare a standard solution containing D829g. The material was prepared by synthesis, and certified for identity and purity by NMIA. 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 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- androsterone- β-glucuronic acid 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 and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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 882 ± 23 μg of anhydrous androsterone- β-glucuronic acid (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.
3 November 2022.

This report supersedes any issued prior to 3 November 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:	Waters alliance 2695
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (65:35 v/v) The aqueous phase was buffered at pH 2.2 using 1% formic acid
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 ampoules in duplicate, March 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 ^{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 ≈ 84% [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.1% [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ 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 at pH 2.2 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 = 0.03% (10 sub samples in duplicate, May 2019)
Karl Fischer analysis:		Moisture content 10.2% mass fraction (May 2019)
Thermogravimetric analysis:		Volatiles content 9.7% and non-volatile residue 1.5 % mass fraction (June 2019)

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 GCMSMS-7000C	
Column:	HP Ultra 1, 25 m × 0.22 mm I.D. × 0.11 μm	
Program:	115 °C (0.8 min), 90°C/min to 180°C, 5°C/min to 190°C, 3°C/min to 230°C, 10°C/min to 265°C, 30°C/min to 320°C (4 min)	
Injector:	250 °C,	Split ratio: 15/1
Transfer line temp:	300 °C	Carrier: Helium
Scan range:	50-700 *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 (13.9 min): 438 (M^+, 53), 423 (100), 333 (57), 243 (35), 182 (39), 169 (69), 73 (85) *m/z*

ESI-MS:

Instrument:	Micromass Quatro LC Micro
Operation:	Negative ion mode, direct infusion at 5 μL/min
Ionisation:	ESI spray voltage at 2.5 kV negative ion
EM voltage:	650 V
Cone voltage:	40 V
Peak:	469.3 $(M\text{-}H^+)^-$ *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 0.81-0.90 (1H, m), 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 (1H, 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 (49.0 ppm)
Spectral data:	δ 11.9, 14.2, 21.2, 22.7, 29.3, 32.1, 32.9, 33.4, 36.4, 36.7, 37.0, 40.3, 49.1, 52.9, 55.7, 73.2, 74.8, 75.4, 76.6, 77.6, 103.0, 172.7, 224.2 ppm

Melting point: 132-153 °C

Microanalysis:

Found:	C = 55.7%; H = 7.9% (June 2019)
Calculated:	C = 57.2%; H = 8.5% (Calculated for $C_{25}H_{34}D_4O_8$ + 10.3% water)
Calculated:	C = 56.4%; H = 8.4% (Calculated for $C_{25}H_{34}D_4O_8$ + 10.3% water + 1.5% NaCl)