Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

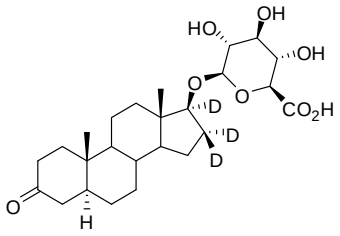
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D573b: d_3-5α-Dihydrotestosterone-β-glucuronide (free acid)

Report ID: D573b.2023.01 (Ampouled 201026)

Chemical Formula: $C_{25}H_{35}D_3O_8$

Molecular Weight: 469.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
10-S-06	Not available	937 μg

IUPAC name: (5α,17β)-3-Oxo(16,16,17-$^2H_3$)androstan-17-yl β-D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 15 May 2028, 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 D573b. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d_3-5α-dihydrotestosterone-β-glucuronide (free acid). d_2-, d_1- and d_0-5α-Dihydrotestosterone-β-glucuronide (free acid) are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_3, d_2 and d_1) and d_0-5α-dihydrotestosterone-β-glucuronide (free 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.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent such as methanol. This will transfer approximately 937 μg of anhydrous 5α-dihydrotestosterone-β-glucuronide (free acid) (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.
18 May 2023.

This report supersedes any issued prior to 18 May 2023.

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:	X-Bridge C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli Q water containing 0.05% TFA; B = Acetonitrile containing 0.05% TFA 0-13 min 33% B; 13-14 min 33-80% B; 14-18 min 80% B; 18-19 min 88-33% B. 19-25 min 33% B
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.02% (7 ampoules in duplicate, November 2020)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, May 2023)

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_3-5α-dihydrotestosterone-β-glucuronide (free acid). d_2-, d_1- and d_0- 5α-dihydrotestosterone-β-glucuronide (free acid) are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_3, d_2 and d_1) and d_0- 5α-dihydrotestosterone-β-glucuronide (free 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 ≈ 95% [= $d_3/(d_3+d_2+d_1+d_0)$ x 100]	
	d_0 < 2.4% [= $d_0/(d_3+d_2+d_1+d_0)$ x 100]	
HPLC:	Instrument:	Waters or Shimadzu Binary pump
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/Milli Q water (33:67) 0.05% TFA was present in both aqueous and organic phases.
	Flow rate:	1 mL/min
	Detector:	Waters ELSD 2424 or Shimadzu ELSD LT-II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.11% (10 sub samples in duplicate, January 2011)
	Re-analysis:	Mean = 99.6%, s = 0.05% (5 sub samples in duplicate, November 2013)
	Re-analysis:	Mean = 99.1%, s = 0.03% (5 sub samples in duplicate, December 2016)
Thermogravimetric analysis:		Non volatile residue 0.34 % mass fraction. The volatile content, organic solvents and/or water, could not be accurately determined using this technique (April 2011)
Karl Fischer analysis:		Moisture content 3.5% mass fraction (April 2011) Moisture content 3.7% mass fraction (November 2013) Moisture content 3.6% mass fraction (November 2016)

Spectroscopic and other characterisation data

ESI -MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 10 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	698 V
	Cone voltage:	35 V
	Peak:	468 ($M\text{-}H^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Tetrahydrofuran (THF) and 2,6-Di-*tert*-butyl-4-methylphenol (BHT)
TLC:	Conditions:	Kieselgel 60F_{254}. Chlorofrom/methanol (2/1) Single spot observed, R_f = 0.3-0.4. Visualisation with vanillin.
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm-1, KBr powder
	Peaks:	3308, 2931, 2845, 1717, 1437, 1409, 1373, 1254, 1188, 1089, 1062, 1021, 935, 679, 467 cm^{-1}.
^{1}H NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.70 (1H, m), 0.75 (3H, s), 0.84 (1H, m), 0.92 (1H, m), 0.97 (3H, s), 1.10 (1H, ddd, *J* = 12.8, 12.8, 3.9 Hz), 1.15 (1H, t, *J* = 12.4 Hz), 1.20-1.48 (7H, m), 1.51(1H, m), 1.61 (1H, m), 1.85-1.95 (3H, m), 2.08 (1H, m), 2.29 (1H, t, *J* = 14.5 Hz), 2.40 (1H, ddd, *J* = 14.7. 14.7, 6.7 Hz), 2.95 (1H, t, *J* = 8.0 Hz), 3.13 (1H, t, *J* = 9.0 Hz), 3.29 (1H, t, *J* = 9.3 Hz), 3.54 (1H, d, *J* = 9.7 Hz), 4.24 (1H, d, *J* = 7.9 Hz), 4.9-5.1 (2H, s, br) ppm THF was observed at 0.5% mass fraction, BHT at 1.0% mass fraction and an unidentified steroid estimated at 2.6% mass fraction was observed in the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	150 MHz
	Solvent:	DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 11.1, 11.4, 20.5, 22.7, 28.3, 30.9, 34.8, 35.3, 36.8, 37.7, 37.9, 42.6, 44.2, 46.0, 50.1, 53.2, 71.5, 73.4, 75.7, 76.1, 103.5, 170.4, 210.5 ppm
Melting point:		221-222 °C
Microanalysis:	Found:	C = 61.9%; H = 8.7% (February 2011)
	Calculated:	C = 63.9%; H = 8.8% (Calculated for $C_{25}H_{35}D_3O_8$)