Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

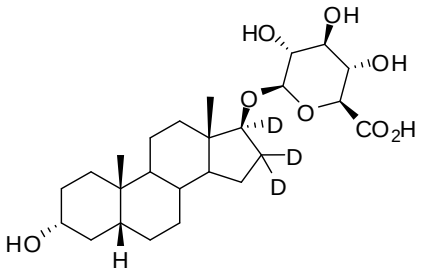
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S011: d_3-5β-Androstane-3α,17β-diol-17-O-β-glucuronic acid

Report ID: S011.2020.03

Chemical Formula: $C_{25}H_{37}D_3O_8$

Molecular Weight: 471.6 g/mol



Property value

Batch No.	CAS No.	Purity estimate by ELSD
11-S-08	Not available	99.4 ± 0.1%

Synonyms: d3-3α-Hydroxy-5β-androstan-17β-yl-β-D-glucopyranosiduronic acid
d3-17β-(β-D-glucopyranuronosyloxy)-5β-androstan-3α-ol.

Expiration of certification: The property values are valid till 23 April 2025, 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 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

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.

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: 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 1-2 mg 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.
16 November 2022

This report supersedes any issued prior to 16 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

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 evaporative light scattering detection (ELSD), 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_3-5β-androstane-3α,7β-diol-17-*O*-β-glucuronic acid. d_2-, d_1- and d_0-5β-Androstane-3α,17β-diol-17-*O*-β-glucuronic 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- d_3-5β-androstane-3α,17β-diol-17-*O*-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: $d_3 \approx 95\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0)$ x 100]
$d_0 < 0.8\%$ [= $d_0/(d_3 + d_2 + d_1 + d_0)$ x 100]
[from SIM analysis of the precursor, d3-testosterone]

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (35:65) 0.5% Formic acid was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Waters 2424 ELS detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.1% (7 sub samples in duplicate, January 2013)
	Re-analysis:	Mean = 99.9%, s = 0.1% (5 sub samples in duplicate, December 2015)
	Re-analysis:	Mean = 100.0%, s = 0.02% (5 sub samples in duplicate, April 2017)
	Re-analysis:	Mean = 99.4%, s = 0.09% (5 sub samples in duplicate, April 2020)
Thermogravimetric analysis:		Volatile content 3.0% and non volatile residue 1.1% mass fraction (February 2013)
Karl Fischer analysis:		Moisture content 2.6% mass fraction (January 2013) Moisture content 5.1% mass fraction (December 2015) Moisture content 5.0% mass fraction (December 2016)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro		
	Column:	X-Bridge C-18, 100 mm × 2.1 mm I.D. × 3.5 μm		
	Column temp:	45 °C		
	Solvent system:	2% Formic acid in MilliQ water [10%], Methanol [60% v/v], MilliQ water [30% v/v]		
	Flow rate:	0.2 mL/min		
	Sample prep:	50 μg/g in MeOH/MilliQ water (25:75)		
	Injection volume:	30 μL		
	Ionisation mode:	Electrospray negative ion		
	Capillary voltage:	3 kV Cone voltage: 35 V		
	Source temp:	130 °C	Desolvation gas temperature:	350 °C
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate:	762 L/hr
	The retention time of d_3-5β-androstane-3α,17β-diol-17-β-glucuronic acid is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.			
	Parent (10.9 min):	470 ($M\text{-}H^+$) *m/z*		
GC-MS:	Instrument:	Agilent 6890/5973		
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm		
	Program:	180 °C, 3 °C/min to 240 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C		
	Injector:	260 °C		
	Transfer line temp:	300 °C		
	Carrier:	Helium, 1.0 mL/min		
	Split ratio:	14/1		
	The free steroid was liberated upon treatment with β-glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA. The retention time of the *bis*-TMS derivative of d_3-5β-androstan-3α, 17β-diol 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 (9.8 min):	439 (M^+, 1), 424 (3), 349 (12), 259 (42), 244 (36), 215 (18), 202 (19), 176 (4), 163 (8), 147 (7), 131 (45), 119 (21), 107 (19), 93 (20), 81 (22), 73 (100) *m/z*		
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (2/1) Single spot observed, R_f = 0.71. Visualisation with vanillin		
IR:	Instrument:	Biorad FTS3000MX FT-IR		
	Range:	4000-400 cm^{-1}, KBr powder		
	Peaks:	3465, 3327, 2930, 2868, 2225, 2134, 1726, 1682, 1450, 1373, 1258, 1233, 1187, 1092, 1051, 1024, 998 cm^{-1}		
^{1}H NMR:	Instrument:	Bruker Avance III-400		
	Field strength:	400 MHz		
	Solvent:	MeOH-d_4 (3.31 ppm)		
	Spectral data:	δ 0.82 (3H, s), 0.95 (3H, s), 0.99-1.64 (16H, m), 1.71-2.00 (4H, m), 3.21 (1H, dd, *J* = 7.9, 9.2 Hz), 3.35 (1H, t, *J* = 9.0 Hz), 3.48-3.58 (2H, m), 3.74 (1H, d, *J* = 9.7 Hz), 4.37 (1H, d, *J* = 7.8 Hz) ppm		
		Dimethyl sulfoxide estimated at 0.7% mass fraction was observed in the ^{1}H NMR.		
^{13}C NMR:	Instrument:	Bruker Avance III-400		
	Field strength:	101 MHz		
	Solvent:	MeOH-d_4 (49.0 ppm)		
	Spectral data:	δ 12.0, 21.5, 23.9, 24.1, 27.2, 28.2, 31.2, 35.8, 36.6, 37.1, 37.2, 38.8, 42.1, 43.6, 44.3, 52.2, 72.4, 73.2, 75.1, 76.7, 77.6, 105.1, 172.9 ppm		
Melting point:		220 °C decomposition		
Microanalysis:	Found:	C = 60.7%; H = 8.6% (February, 2013)		
	Calculated:	C = 63.7%; H = 8.6% (Calculated for $C_{25}H_{37}D_3O_8$)		