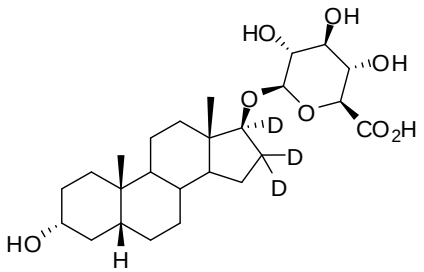
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S011b: d_3-5β-Androstane-3α,17β-diol-17-O-β-glucuronic acid

Report ID: S011b.2024.01

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

Molecular Weight: 471.6 g/mol



Property value

Batch No.	CAS No.	Purity estimate by CAD
24-S-05	Not available	81.1 ± 2.1%

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 25 June 2027, 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

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.

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 CAD detection on ten 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.
26 September 2024
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 and charged aerosol detection (ELSD/CAD), 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,17β-diol-17-*O*-β-glucuronic acid. d_4-, 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_4, 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.

Isotopic Purity: $d_3 \approx 92\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0)$ x 100]

$d_0 < 0.2\%$ [= $d_0/(d_3 + d_2 + d_1 + d_0)$ x 100]

[from SIM analysis of d_3-5β-androstane-3α,17β-diol-17-*O*-β-glucuronic acid]

HPLC:	Instrument:	Thermo Scientific Vanquish
	Column:	ACE Excel Super C18 C18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40) 0.2% Formic acid was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Vanquish detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 76.3%, s = 0.2% (10 sub samples in duplicate, June 2024)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE Excel Super C18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40) 0.2% Formic acid was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 95.9%, s = 0.1% (10 sub samples in duplicate, June 2024)
Thermogravimetric analysis:		Volatile content 5.3% and non volatile residue 0.3% mass fraction (July 2024)
Karl Fischer analysis:		Moisture content 5.2% mass fraction (July 2024)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Shimadzu
	Column:	ACE Excel Super C18 C18, 5 μm (4.6 mm x 150 mm)
	Column temp:	40 °C
	Solvent system:	Methanol/MilliQ water (60:40) 0.2% Formic acid was present in the aqueous phase.
	Flow rate:	1 mL/min
	Sample prep:	1 μg/g in MeOH
	Injection volume:	10 μL
	Ionisation mode:	Electrospray negative ion
	Interface voltage:	-3.0 kV
	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 (16.8 min):	470 $[M-H]^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (2/1) Single spot observed, R_f = 0.7. 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), 3.20 (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
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 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, 172.6 ppm
Melting point:		220 °C decomposition
Microanalysis:	Found:	C = 60.8%; H = 8.4% (August 2024)
	Calculated:	C = 63.7%; H = 8.6% (Calculated for $C_{25}H_{37}D_3O_8$)