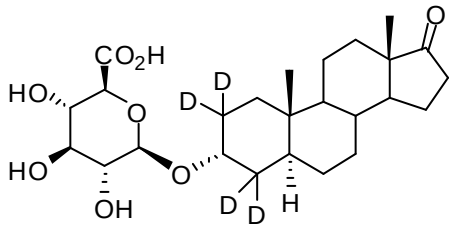
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

Report ID: S012.2018.01
Compound Name: **d_5-5β-Androstane-3α,17β-diol-3-*O*-β-glucuronic acid**
Description: White solid
Collection Number: S012 | Batch Number: 11-S-09
Chemical Formula: $C_{25}H_{35}D_5O_8$ | Molecular Weight: 473.6
CAS Number: NA | Release date: 18th February 2013
Structure:



Synonyms: d_5-(3α,5β,17β)-17-Hydroxyandrostan-3-yl β-D-glucopyranosiduronic acid
d_5-17β-Hydroxy-5β-androstan-3α-yl β-D-glucopyranosiduronic acid

Purity (mass fraction): 93.4 ± 1.7% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques, by subtraction from 100% of total impurities by HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis, and ^{1}H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

The main component of this material is d_5-5β-androstane-3α,17β-diol-3-*O*-β-glucuronic acid. d_4-, d_3-, d_2-, d_1- and d_0-5β-androstane-3α,17β-diol-3-*O*-β-glucuronic acid are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-5β-androstane-3α,17β-diol-3-*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: $d_5 \approx 93\%$ [$= d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 0.2\%$ [$= d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
[from SIM analysis of the precursor, d_5-etiocholanolone]

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm) (2013) X-Bridge C-18, 5 μm (4.6 mm x 150 mm) (2018)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (65:35) 0.5% Formic acid was present in the aqueous phase. (2013) Methanol/MilliQ water (61:39) 0.5% Formic acid was present in the aqueous phase. (2018)
	Flow rate:	1 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of main component :	
	Initial analysis:	Mean = 99.4%, s = 0.01% (10 sub samples in duplicate, January 2013)
	Re-analysis:	Mean = 99.5%, s = 0.16% (7 sub samples in duplicate, June 2018)
Thermogravimetric analysis:		Volatile content 3.4% and non volatile residue 1.5% mass fraction (February 2013).
Karl Fischer analysis:		Moisture content 4.0% mass fraction (February 2013) Moisture content 4.7% mass fraction (June 2018)

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:	30 °C	
	Solvent system:	2% Formic acid in MilliQ water [10%], Methanol [60% v/v], MilliQ water [30% v/v]	
	Flow rate:	1 mL/min	
	Sample prep:	50 μg/g in MeOH/MilliQ water (25:75)	
	Injection volume:	30 μL	
	Ionisation mode:	Electrospray negative ion	
	Capillary voltage:	3.0 kV	Cone voltage: 35 V
	Source temp:	130 ºC	Desolvation gas temperature: 350 ºC
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate: 762 L/hr
	The retention time of d_5-5β-androstan-3α, 17β-diol-3-*O*-β-glucuronic acid is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	9.75 min:	472.4 ($M\text{-}H^+$) 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 ºC	Transfer line temp: 300 ºC
	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_5-5β-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):	441 (M^+, 1), 426 (3), 351 (10), 261 (43), 246 (41), 235 (7), 220 (24), 199 (16), 175 (4), 160 (8), 147 (8), 129 (56), 116 (13), 107 (12), 101 (11), 93 (16), 81 (16), 73 (100) m/z	
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (2/1) Single spot observed, R_f = 0.86. Visualisation with vanillin	
IR:	Instrument:	Biorad FTS3000MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	3551, 3441, 3342, 2980, 2932, 2922, 2897, 2864, 2203, 2122, 1722, 1448, 1375, 1336, 1254, 1215, 1168, 1123, 1062, 1050, 1017, 996, 944, 925, 695 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-400	
	Field strength:	400 MHz	Solvent: CD_3OD (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.96 (3H, s), 0.94-1.18 (4H, m), 1.19-1.32 (3H, m), 1.37-1.52 (6H, m), 1.59 (1H, m), 1.81-2.02 (4H, m), 3.19 (1H, dd, *J* = 7.9, 9.2 Hz), 3.38 (1H, t, *J* = 9.0 Hz), 3.52 (1H, t, *J* = 9.6 Hz), 3.57 (1H, t, *J* = 8.7 Hz) 3.78 (1H, d, *J* = 9.7 Hz), 4.45 (1H, d, *J* = 7.8 Hz) ppm	
^{13}C NMR:	Instrument:	Bruker Avance III-400	
	Field strength:	101 MHz	Solvent: CD_3OD (49.0 ppm)
	Spectral data:	δ 11.7, 21.5, 23.9, 24.3, 27.2, 28.1, 30.7, 35.8, 36.2, 37.3, 38.2, 42.1, 43.4, 44.2, 52.4, 73.2, 74.8, 76.6, 77.5, 82.6, 102.5, 172.6 ppm	
Melting point:		208-210 °C decomposition	
Microanalysis:		Found: C = 59.6%; H = 8.6% (February, 2013) Calc: C = 63.4%; H = 8.6% (Calculated for $C_{25}H_{35}D_5O_8$)	

Expiration of certification
The property values are valid till 14th June 2021, i.e. three 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.

The long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of three years from the date of re-certification.

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years.

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.

Recommended storage
When not in use this material should be stored at or below 4 ºC in a closed container, protected from ambient moisture and light.

Intended use
For use as an internal standard only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 17 August, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 17th August 2018.