



REFERENCE MATERIAL ANALYSIS REPORT

Report ID: S011.2017.01

Compound Name: **d₃-5β-Androstane-3α,17β-diol-17-O-β-glucuronic acid**

Description: White solid

Collection Number: S011

Chemical Formula: C₂₅H₃₇D₃O₈

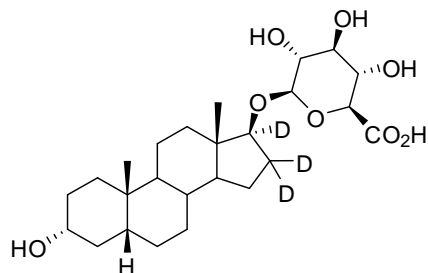
CAS Number: NA

Structure:

Batch Number: 11-S-08

Molecular Weight: 471.6

Release date: 26th February 2013



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

Purity (mass fraction): 93.0 ± 2.0% (95% coverage interval)

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

The main component of this material is d₃-5β-androstane-3α,17β-diol-17-β-glucuronic acid. d₂-, d₁- and d₀- 5β-Androstane-3α,17β-diol-17-β-glucuronic acid are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d₃, d₂ and d₁) and d₀- 5β-androstane-3α,17β-diol-17-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: d₃ ≈ 95% [= d₄/(d₄ + d₃ + d₂ + d₁ + d₀) x 100]
d₀ < 0.8% [= d₀/(d₄ + d₃ + d₂ + d₁ + d₀) 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 response of 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)

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)

Accredited for compliance with ISO Guide 34.

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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 d₃-5β-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.

10.9 min: 470 (M-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 d₃-5β-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
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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: CD ₃ OD (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: CD ₃ OD (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)
Calc: C = 63.7%; H = 8.6% (Calculated for C₂₅H₃₇D₃O₈)

Expiration of certification

The property values are valid till 10th April 2020, 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 °C in a closed container in a dry, dark area.

Intended use

For *in vitro* laboratory analysis 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: 11 April, 2017.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 11th April 2017.