Australian Government

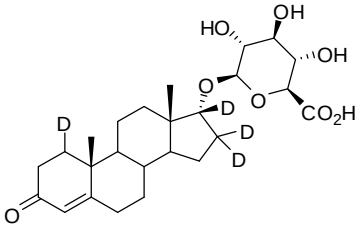
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: S023.2016.01

Compound Name: **d_4-Epitestosterone-17-*O*-β-glucuronic acid**
Collection Number: S023
Chemical Formula: $C_{25}H_{32}D_4O_8$
CAS Number: NA
Description: White solid
Batch Number: 13-S-07
Molecular Weight: 468.6
Release date: 10th October 2013

Structure:



Synonyms: Epitestosterone-1,16,16,17-d_4 glucuronide
d_4-Epitestosterone glucuronoside
d_4-Epitestosterone glucosiduronate
d_4-(17α)-3-Oxoandrost-4-en-17-yl glucopyranosiduronic acid

Purity (mass fraction): 91.0 ± 2.0%

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

The main component of this material is d_4-epitestosterone-17-*O*-β-glucuronic acid. d_3-, d_2-, d_1- and d_0-Epitestosterone-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-epitestosterone-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: $d_4 \approx 96\%$ [$= d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 0.2\%$ [$= d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
[from SIM analysis of the free steroid]

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (55:45) 0.5% Formic was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Shimazu SPD-M20A PDA operating at 246 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.3%, s = 0.05% (7 sub samples in duplicate, August 2013)
	Re-analysis:	Mean = 99.4%, s = 0.03% (5 sub samples in duplicate, August 2016)
Thermogravimetric analysis:		Volatile content 5.6% and non volatile residue < 0.2% mass fraction (September 2016)
Karl Fischer analysis:		Moisture content 5.6% mass fraction (September 2016)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Waters Acquity, UPLC, QBA 119
	Operation:	Negative ion mode, direct infusion at 10 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV positive ion
	Cone voltage:	20 V
	Peak:	467.5 ($M\text{-}H^+$) m/z

GC-MS: The free steroid was liberated upon treatment with β-glucuronidase enzyme (*E. Coli* K12) and derivatised with MSTFA.

Instrument:	Shimadzu GC-2010/GCMS-QP210 plus	
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 retention times of the *bis*-TMS derivative 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 (10.8 min): 436 (M^+, 98), 421 (11), 331 (13), 210 (20), 73 (100) m/z
The silylated compound co-elutes with a derivatised comparison sample of epitestosterone.

IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	2936, 2877, 2157, 1733, 1623, 1434, 1370, 1339, 1253, 1191, 1164, 1061, 1018, 935, 698, 654, 598 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz Solvent: CD_3OD (3.31 ppm)
	Spectral data:	δ 0.77 (3H, s), 0.99 (1H, m), 1.10 (1H, m), 1.24 (3H, s), 1.26 (1H, t, *J* = 12.0 Hz), 1.44-1.71 (5H, m), 1.76-1.84 (2H, m), 1.93 (1H, m), 2.08 (1H, m), 2.26-2.33 (2H, m), 2.44-2.54 (2H, m), 3.18 (1H, dd, *J* = 7.8, 9.2 Hz), 3.36 (1H, t, *J* = 9.1 Hz), 3.52 (1H, t, *J* = 9.1 Hz), 3.72 (1H, d, *J* = 9.8 Hz), 4.28 (1H, d, *J* = 7.8 Hz), 5.71 (1H, s) ppm
		Methanol estimated at 2.8% mass fraction was observed in the ^{1}H NMR (2016)
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	101 MHz Solvent: CD_3OD (49.0 ppm)
	Spectral data:	δ 17.4, 17.8, 21.7, 25.5, 32.8, 33.7, 34.1, 34.7, 37.2, 40.0, 45.8, 50.3, 55.3, 73.2, 74.7, 76.7, 77.6, 102.5, 124.1, 172.7, 175.5, 202.5 ppm
Melting point:		218 °C decomposition
Microanalysis:		Found: C = 62.2%; H = 7.9% (August, 2013) Calc: C = 64.1%; H = 7.8% (Calculated for $C_{25}H_{32}D_4O_8$)

Expiration of certification
The property values are valid till 25th August 2019, 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 demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by HPLC with UV 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 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: 7 October, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 7th October 2016.