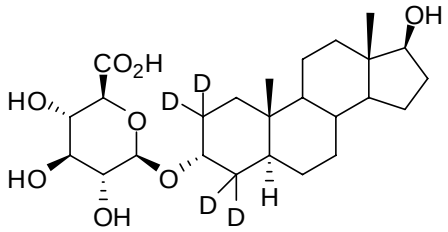
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: S010.2012.01

Compound Name: **d_4-5α-Androstan-3α,17β-diol-3-*O*-β-glucuronic acid**
Description: White solid
Collection Number: S010
Batch Number: 11-S-07
Chemical Formula: $C_{25}H_{36}D_4O_8$
Molecular Weight: 472.6
CAS Number: NA
Release Date: 14th February 2013
Structure:



Synonyms: NA

Purity (mass fraction): 96.3 ± 1.7% (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 ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

The main component of this material is d_4-5α-androstan-3α,17β-diol-3-*O*-β-glucuronic acid. d_3-, d_2-, d_1- and d_0- 5α-Androstan-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_4, d_3, d_2 and d_1) and d_0- 5α-androstan-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: $d_4 \approx 83\%$ [$= d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 0.2\%$ [$= d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
[from SIM analysis of the *bis*-TMS derivatised free steroid]

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler

Column:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
Column oven:	40 °C
Mobile Phase:	Methanol/MilliQ water (60:40) Formic acid (0.5% v/v) was present in the aqueous phase.
Flow rate:	1.0 mL/min
Detector:	Waters 2424 ELS detector

Relative peak area response of main component:
Initial analysis: Mean = 99.8%, s = 0.02% (10 sub samples in duplicate, September 2012)

Thermogravimetric analysis: Volatile content 1.7% and non volatile residue < 0.2% mass fraction (September 2012)

Karl Fischer analysis: Moisture content 2.9% mass fraction (September 2012)

Spectroscopic and other characterisation data

ESI-MS:	Instrument	Micromass Quatro Micro
	Operation:	Negative ion mode, direct infusion at 5 µL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage	20 V
	Peak:	471.5 ($M-H^+$) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (2/1) Single spot observed, R_f = 0.88. Visualisation with vanillin
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3420, 2917, 2868, 2203, 2109, 1716, 1446, 1266, 1218, 1161, 1056, 1016 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	600 MHz Solvent: CD_3OD (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.78 (1H, m), 0.83 (3H, s), 0.89-1.04 (3H, m), 1.14-1.32 (4H, m), 1.36-1.48 (4H, m), 1.55-1.61 (2H, m), 1.63-1.68 (2H, m), 1.81 (1H, ddd, *J* = 12.2, 3.1, 3.1 Hz), 1.96 (1H, m), 3.24 (1H, dd, *J* = 7.9, 9.3 Hz), 3.38 (1H, t, *J* = 9.1 Hz), 3.53 (1H, t, *J* = 9.6 Hz) , 3.55 (1H, t, *J* = 8.7 Hz), 3.76 (1H, d, *J* = 9.8 Hz), 3.93, (1H, s), 4.37 (1H, d, *J* = 7.8 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	151 MHz Solvent: CD_3OD (49.0 ppm)
	Spectral data:	δ 11.7, 11.9, 21.5, 24.3, 25.8, 29.5, 30.6, 32.9, 33.5, 34.5, 36.9, 38.1, 40.3, 44.1, 52.5, 55.8, 73.2, 74.8, 75.5, 76.6, 77.7, 82.6, 103.1, 172.7 ppm
Melting point:		225-228 ºC
Microanalysis:		Found: C = 62.1%; H = 8.7%; (September, 2012) Calc: C = 61.7%; H = 8.7%; (Calculated for $C_{25}H_{36}D_4O_8$ + 3% water)

Expiration of certification
The property values are valid till 3rd September 2015, i.e. 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.

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 certification. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

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 ten randomly selected 1-2 mg sub samples of the material. The material was judged to be 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: 14 February, 2013.

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