



REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D573b.2011.01

Compound Name: **d₃-5 α -Dihydrotestosterone- β -glucuronide (free acid)**

Description: Off white solid

Collection number: D573b

Chemical Formula: C₂₅H₃₅D₃O₈

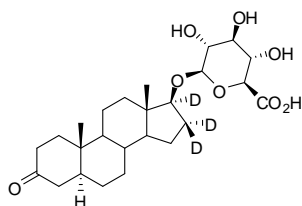
CAS Number: NA

Structure:

Batch number: 10-S-06

Molecular Weight: 469.6

Batch production completed: January 2011



Synonyms: d₃-5 α -Androstane-17 β -ol-3-one glucuronide
d₃- (5 α ,17 β)-3-Oxoandrostane-17-yl β -D-glucopyranosiduronic acid
d₃-3-Oxo-5 α -androstane-17 β -yl glucosiduronic acid
d₃-17 β -Hydroxy-5 α -androstane-3-one glucuronide
d₃-5 α -Dihydrotestosterone glucuronide
d₃-Dihydrotestosterone 17-glucuronide
d₃-Dihydrotestosterone 17 β -O-glucuronide

Purity (mass fraction): 91.7 \pm 2.1% (95% coverage interval)

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 analysis. Supporting evidence is provided by headspace GC-MS analysis of occluded solvents and elemental microanalysis.

The main component of this material is d₃-5 α -dihydrotestosterone- β -glucuronide. d₂-, d₁- and d₀-5 α -dihydrotestosterone- β -glucuronide are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d₃, d₂ and d₁) and d₀-5 α -dihydrotestosterone- β -glucuronide in the material.

The isotopic purity of this material has not been accurately determined. The stated d₃ purity is an estimate only. A fragment ion equal to the molecular weight of d₀ is observed at 1.8% of the d₃ peak. This material should be considered for use as an internal standard only.

Isotopic Purity: d₃ \approx 95% [= d₃ / (d₃ + d₂ + d₁ + d₀) x 100]

d₀ \approx 2.4%

(determined by SIM analysis of d₃-5 β -DHT obtained after acid cleavage of the glucuronide)

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler Column: X-Bridge C-18, 5 μ m (4.6 mm x 150 mm)

Column oven: Ambient

Mobile Phase: Acetonitrile/MilliQ water (33:67)
0.05% TFA was present in both aqueous and organic phases.

Flow rate: 1 mL/min

Detector: Shimadzu ELSD LT-II

Retention time: 8.0 min

Relative peak area response of main component:

Initial analysis: Mean = 99.6%, s = 0.11% (10 sub samples in duplicate, January 2011)

Thermogravimetric analysis: Non volatile residue 0.34 % mass fraction. The volatile content (e.g. organic solvents and/or water) could not be accurately determined using this technique. (April 2011).

Karl Fischer analysis: Moisture content 3.5% mass fraction (April 2011)

Spectroscopic and other characterisation data

ESI -MS:	Instrument: Micromass Quatro LC Micro Operation: Negative ion mode, direct infusion at 10 µL/min Ionisation: ESI spray voltage at 3.0 kV negative ion EM voltage: -698 V Cone voltage: 35 V Peak: 468 (M-H ⁺) m/z
HS-GC-MS:	Instrument: Agilent 6890/5973/G1888 Column: DB-624, 30 m x 0.25 mm I.D. x 1.4 µm Program: 50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min) Injector: 150 °C Transfer line temp: 280 °C Carrier: Helium, 1.2 mL/min Split ratio: 50/1 Solvents detected: Tetrahydrofuran (THF) and 2,6-Di- <i>tert</i> -butyl-4-methylphenol (BHT)
TLC:	Conditions: Kieselgel 60F ₂₅₄ . Chloroform/methanol (2/1) Single spot observed, R _f = 0.33. Visualisation with vanillin
IR:	Instrument: Biorad FTS3000MX FT-IR Range: 4000-400 cm ⁻¹ , KBr powder Peaks: 3308, 2931, 2845, 1717, 1437, 1409, 1373, 1254, 1188, 1089, 1062, 1021, 935, 679, 467 cm ⁻¹
¹ H NMR:	Instrument: Bruker Avance DMX-600 Field strength: 600 MHz Solvent: DMSO-d ₆ (2.50 ppm) Spectral data: δ 0.70 (1H, m), 0.75 (3H, s), 0.84 (1H, m), 0.92 (1H, m), 0.97 (3H, s), 1.10 (1H, ddd, J = 12.8, 12.8, 3.9 Hz), 1.15 (1H, t, J = 12.4 Hz), 1.20-1.48 (7H, m), 1.51 (1H, m), 1.61 (1H, m), 1.85-1.95 (3H, m), 2.08 (1H, m), 2.29 (1H, t, J = 14.5 Hz), 2.40 (1H, ddd, J = 14.7, 14.7, 6.7 Hz), 2.95 (1H, t, J = 8.0 Hz), 3.13 (1H, t, J = 9.0 Hz), 3.29 (1H, t, J = 9.3 Hz), 3.54 (1H, d, J = 9.7 Hz), 4.24 (1H, d, J = 7.9 Hz), 4.9-5.1 (2H, s, br) ppm THF was observed at 0.5% mass fraction, BHT at 1.0% mass fraction and an unidentified steroid estimated at 2.6% mass fraction was observed in the ¹ H NMR
¹³ C NMR:	Instrument: Bruker Avance DMX-600 Field strength: 150 MHz Solvent: DMSO-d ₆ (39.5 ppm) Spectral data: δ 11.1, 11.4, 20.5, 22.7, 28.3, 30.9, 34.8, 35.3, 36.8, 37.7, 37.9, 42.6, 44.2, 46.0, 50.1, 53.2, 71.5, 73.4, 75.7, 76.1, 103.5, 170.4, 210.5 ppm
Melting point:	221-222 °C
Microanalysis:	Found: C = 61.9%; H = 8.7% (February, 2011) Calc: C = 63.9%; H = 8.8% (Calculated for C ₂₅ H ₃₅ D ₃ O ₈)

Comment [NMI 1]: Possibly ddd

Expiration of certification

The property values are valid till January 2014, 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 °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: 1 May, 2013.

Characterisation data and property values specified in this report were first issued on 6th May 2011.



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