



REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D678.2012.02

Compound Name: **5 $\alpha$ -Androstane-3 $\beta$ , 17 $\beta$ -diol-3-glucuronic acid hydrate**

Description: Colourless powder

Collection No.: D678

Chemical Formula: C<sub>25</sub>H<sub>40</sub>O<sub>8</sub>

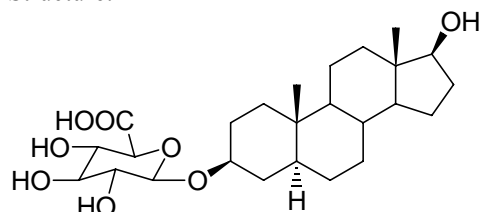
CAS Number: N/A

Structure:

Batch No: 00-S-19

Formula Weight: 468.6

Batch production completed: December 2000



Purity (mass fraction %): 88.6 ± 2.0% (95% coverage interval)

Purity estimate obtained by subtraction from 100% of total impurities by HPLC with ELSD detection and Karl Fischer moisture analysis. Supporting evidence is provided by elemental microanalysis and <sup>1</sup>H NMR.

**Note: 1 mg of D678 is equivalent to approximately 886 μg of the anhydrous glucuronic acid.**

HPLC:	Column:	Alltima C18, 5 μm (4 mm × 150 mm)
	Mobile Phase:	Ammonium acetate buffer (20 mM, pH 4.2) / acetonitrile (72/28)
	Flow Rate:	0.8 mL/min
	Detector:	ELSD
	Retention time:	6.7min
Relative peak area response of main component:		
	Initial analysis:	Mean = 99.9%, s = 0.1% (10 sub samples in duplicate, February 2001)
	Re-analysis:	Mean = 99.99%, s = 0.001% (2 sub samples in duplicate, August 2003)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, May 2007)
	Re-analysis:	Mean = 100.0%, s = 0.02% (5 sub samples in duplicate, April 2012)
ESI-MS:	Instrument	TSQ-700
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH <sub>4</sub> OAc, pH 4.2: MeOH (1:1).
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range m/z 100-600.
	Major ion:	486.5 ([MNH <sub>4</sub> ] <sup>+</sup> , 100%)
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH <sub>4</sub> OAc: MeOH (1:1).
	Scan:	5 scans of 5 seconds, dwell time 1 ms per ion, scan range m/z 100-600.
	Major ion:	466.7 ([M-H] <sup>-</sup> , 100%) m/z
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . CHCl <sub>3</sub> /MeOH/H <sub>2</sub> O (70:30:2) Single spot observed, R <sub>f</sub> = 0.19 (3 samples)
IR:	Instrument:	Biorad FT-IR
	Range:	4000-400 cm <sup>-1</sup> , KBr pellet.
	Peaks:	3512, 2930, 1738, 1607, 1237, 1051 cm <sup>-1</sup>
<sup>1</sup> H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz
	Key spectral data:	δ 0.85 (3H, s), 0.72 (3H, s), 4.45 (1H, d) ppm
<sup>13</sup> C NMR:	Instrument:	Bruker DMX600
	Field strength:	150 MHz
	Spectral data:	δ 171.7, 101.7, 81.5, 78.8, 76.5, 75.6, 73.8, 72.2, 55.0, 51.4, 45.1, 43.1, 37.3, 37.1, 35.9, 35.8, 34.4, 31.9, 29.6, 29.4, 28.9, 23.3, 21.0, 11.7, 10.7 ppm

Microanalysis: Found: C = 60.6%; H = 8.9% (January 2001)  
Calc: C = 60.6%; H = 8.8% (Calculated for 1.5 hydrate)

Karl Fischer analysis: Moisture content c.a. 11% mass fraction. (May 2007 and April 2012)

#### Expiration of certification

The property values are valid till 13<sup>th</sup> April 2017, i.e. five 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/ampoules 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 5 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 on 5 randomly selected 1 mg 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: 2 August, 2012.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 2<sup>nd</sup> August 2012.



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