



## REFERENCE MATERIAL ANALYSIS REPORT

**Report ID: D820.2011.02 (Ampouled 050629)**This batch of ampoules was prepared from the bulk material on 29<sup>th</sup> June 2005Compound Name: **18a-Homo-17 $\alpha$ -ethyl-5 $\alpha$ -estrane-3 $\alpha$ , 17 $\beta$ -diol**

Description: White powder

Collection Number D820

Chemical Formula: C<sub>21</sub>H<sub>36</sub>O<sub>2</sub>

CAS Registry Number: N/A

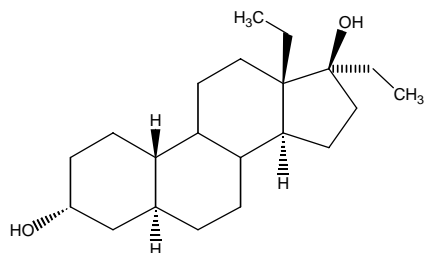
Batch Number: 03-S-05

Molecular Weight: 320.5

Batch production completed: June 2003

Metabolite of norbolethone

Structure:

Synonym: 18a-Homo-19-nor-17 $\alpha$ -pregnane-3 $\alpha$ , 17 $\beta$ -diol13 $\beta$ , 17 $\alpha$ -Diethyl-5 $\alpha$ -gonane-3 $\alpha$ , 17 $\beta$ -diol

**The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D820. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 0.986  $\pm$  0.014 mg of anhydrous**

**18a-homo-17 $\alpha$ -ethyl-5 $\alpha$ -estrane-3 $\alpha$ , 17 $\beta$ -diol. The uncertainty is stated at the 95% coverage interval.**

GC-FID: Instrument: Agilent 6890N  
Column: HP-1, Capillary, 29.9 m  $\times$  0.32 mm I.D.  $\times$  0.25  $\mu$ m  
Program: 180  $^{\circ}$ C (1 min), 40  $^{\circ}$ C/min to 250  $^{\circ}$ C (8 min), 40  $^{\circ}$ C/min to 300  $^{\circ}$ C (3 min)  
Injector: 250  $^{\circ}$ C Detector Temp: 320  $^{\circ}$ C  
Carrier: Helium Split ratio: 20/1  
Relative peak area response of main component:  
Initial analysis: 99.8%, s = 0.02% (7 sub samples in duplicate, June 2003)  
Re-analysis: 98.3%, s = 0.08% (5 ampoules in duplicate, June 2006)  
Re-analysis: 98.9%, s = 0.02% (5 ampoules in duplicate, July 2007)  
Re-analysis: 98.9%, s = 0.02% (5 ampoules in duplicate, July 2008)  
Current re-analysis: 98.9%, s = 0.01% (5 ampoules in duplicate, July 2011)

**The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.**

Purity estimate 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 GC-FID and thermogravimetric analysis. Supporting evidence is provided by elemental microanalysis.

GC-FID: Instrument: HP5890  
Column: Zebron ZB Capillary, 30 m  $\times$  0.32 mm I.D.  $\times$  0.25  $\mu$ m  
Program: 180  $^{\circ}$ C (1 min), 40  $^{\circ}$ C/min to 250  $^{\circ}$ C, (10 min), 40  $^{\circ}$ C/min to 300  $^{\circ}$ C (2 min)  
Injector: 250  $^{\circ}$ C Detector Temp: 325  $^{\circ}$ C  
Carrier: Helium Split ratio: 15/1  
Relative peak area response of main component:  
Initial analysis: 99.8%, s = 0.02% (7 sub samples in duplicate, June 2003)  
Re-analysis: 98.3%, s = 0.08% (5 sub samples in duplicate, June 2005)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.3% mass fraction (March 2005)

### Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	Zebron ZB 30 m x 0.25 mm I.D. x 0.25 $\mu$ m
	Program:	200 °C (5 min), 10 °C /min to 250 °C (5 min), 40 °C/min to 300 °C (2 min)
	Injector:	280 °C Transfer line temp: 280 °C
	Carrier:	Helium Split ratio: 20/1
	Bis-trimethylsilyl derivative:	
	Instrument:	HP6890/5973
	Column:	Ultra 1, 17 m x 0.20 mm ID x 0.11 $\mu$ m
	Program:	189 °C, 3 °C /min to 240 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C (2 min).
	Injector:	250 °C Transfer line temp: 300 °C
	Carrier:	Helium Split ratio: 14/1
	The retention times of the parent material and its <i>bis</i> -TMS derivative are reported along with the major peaks in their mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (12.8 min):	320 ( $M^+$ , 12), 302 (10), 291 (100), 273 (66), 255 (56), 231 (72), 230 (79), 217 (55), 215 (42), 201 (40), 121 (59), 57 (67) m/z
	TMS (13.6 min):	435 (59), 255 (11), 157 (100), 144 (44), 73 (44) m/z
IR:	Instrument:	Biorad FTS3000MX
	Range:	4000-400 $\text{cm}^{-1}$ , KBr
	Peaks:	3389, 2921, 1460, 1442, 1169, 1069, 972, 932, 870 $\text{cm}^{-1}$
$^1\text{H}$ NMR:	Instrument:	Bruker DPX2-300
	Field strength:	300 MHz Solvent: $\text{CDCl}_3$ (7.26 ppm)
	Key spectral data:	$\delta$ 0.95 (2 x 3H, 2 x t), 4.08 (1H, m) ppm
$^{13}\text{C}$ NMR:	Instrument:	Bruker DPX2-300
	Field strength:	75 MHz Solvent: $\text{CDCl}_3$ (77.16 ppm)
	Spectral data:	$\delta$ 7.6, 9.9, 20.2, 23.0, 23.7, 25.6, 27.7, 29.8, 30.8, 33.0, 33.7, 34.4, 36.2, 40.7, 42.0, 47.0, 47.8, 48.0, 51.6, 66.5, 85.5 ppm
Microanalysis:	Found: C = 78.6%, H = 11.2% (August, 2003) Calc: C = 78.7%, H = 11.3% (Calculated for $\text{C}_{19}\text{H}_{26}\text{O}_3$ )	
Melting point:	158-159 °C	

### Expiration of certification

The property values are valid till 4<sup>th</sup> July 2016, 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 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 gas chromatography with flame ionisation detection on 5 randomly selected ampoules 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: 18 July, 2012

Characterisation data and property values specified in this report supersede those in all reports issued prior to 17<sup>th</sup> July 2012.



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