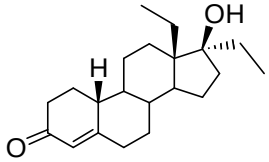
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D825b.2015.01 (Ampouled 140911)
This batch of ampoules was prepared from the bulk material on 11th September 2014.

Compound Name: **Norbolethone**
Collection Number: D825b
Chemical Formula: $C_{21}H_{32}O_2$
CAS Number: 1235-15-0
Structure:



Description: White solid
Batch Number: 06-S-04
Molecular Weight: 316.5
Release Date: July 2006

Synonyms: (17α)-(+/-)-13-Ethyl-17-hydroxy-18,19-dinor-pregn-4-en-3-one
dl-13β,17α-diethyl-17β-hydroxygon-4-en-3-one

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 D825b. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform). This will transfer 977 ± 50 µg of anhydrous norbolethone. The uncertainty is stated at the 95% coverage interval.

Warning: This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.

GC-FID:	Instrument:	Agilent 7890	
	Column:	HP-1MS, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.1%, s = 0.01% (7 ampoules in duplicate, September 2014)	
GC-FID:	Instrument:	Varian CP-3800	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)	
	Injector:	180 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.4%, s = 0.03% (5 ampoules in duplicate, September 2015)	

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

The purity value was obtained from traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID and Karl Fischer analysis. Supporting evidence is provided by elemental microanalysis and ^{1}H NMR.

GC-FID:	Instrument:	HP5890	
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.05%, s = 0.05% (10 sub samples in duplicate, July 2006)	
GC-FID:	Instrument:	Agilent 7890	
	Column:	HP-1MS, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.1%, s = 0.01% (7 sub samples in duplicate, September 2014)	
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (September 2014)	
Thermogravimetric analysis:		Initial volatile content < 0.1% and non volatile residue < 0.2 % mass fraction. Note: Volatile content was based on ^{1}H NMR	
^{1}H NMR Volatile analysis:		Volatile content based on integral for ethyl acetate at 4.1 ppm, compared with norbolethone integral at 5.82 ppm gave 0.76% ethyl acetate mass fraction.	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP5890/5971A	
Column:	ZB-5MS, 26 m x 0.25 mm I.D. x 0.25 μm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (7 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min.	Split ratio: 30/1

Bis-TMS derivative:

Instrument:	HP 6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm	
Program:	180 °C (0.5 min), 12 °C/min to 310 °C (3 min)	
Injector:	260 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 30/1

The retention times of the parent compound and *bis*-TMS derivative are reported along with the major peaks in the mass spectra. The latter are reported as m/z. and (in brackets) as a percentage relative to the base peak.

Parent (9.3 min): 316 (M^+, 59), 287 (19), 269 (13), 245 (53), 229 (29), 110 (45), 91 (57), 85 (61), 57 (100) m/z

Bis-TMS (17.3 min): 460 (M^+, 33), 431 (6), 370 (7), 341 (3), 314 (5), 301 (100), 194 (8), 157 (6), 143 (5), 129 (4), 73 (75) m/z

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (3/1)
Single spot observed, R_f = 0.38. Visualisation with UV at 254 nm

IR:

Instrument:	Biorad FTS300MX FT-IR
Range:	4000-400cm^{-1}, KBr pellet
Peaks:	3611, 3476, 2927, 2860, 1665, 1616, 1454, 1258, 1212, 882cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500	
Field strength:	500 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.77-0.83 (1H, m), 0.95 (3H, t, *J* = 7.25 Hz), 1.0 (3H, t, *J* = 7.45 Hz), 1.03-1.63 (15H, m),1.81-1.87 (3H, m), 1.92-1.98 (1H, m), 2.06-2.12 (1H, m), 2.22-2.32 (3H, m), 2.37-2.44 (1H, m), 2.45-2.50 (1H, m), 5.82 (1H, s) ppm	

^{13}C NMR:

Instrument:	Bruker Gyro-300	
Field strength:	75 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 7.5, 9.9, 20.2, 23.0, 26.4, 26.6, 27.5, 29.8, 31.0, 34.3, 35.5, 36.5, 41.1, 42.5, 47.7, 49.3, 51.0, 85.2, 124.6, 166.7, 199.9 ppm.	

Melting point: 163-168 °C

Microanalysis: Found: C = 79.9 %, H = 10.3 % (June 2006)
Calc: C = 79.7 %, H = 10.2 % (Calculated for $C_{21}H_{32}O_2$)

Expiration of certification
The property values are valid till 30th September 2018, 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 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 been given a shelf life of three years from the date of re-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 GC-FID on seven randomly selected ampoules 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: 13 November, 2015.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 13 November, 2015.