**Australian Government**

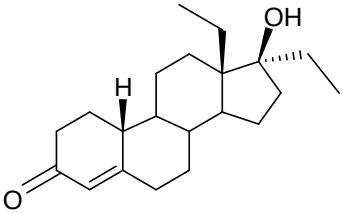
**National Measurement Institute**

## CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

**Report ID: D825c.2018.01 (Ampouled 171012)**
This batch of ampoules was prepared from the bulk material on 12th October 2017.

Compound Name: **Norbolethone**
Collection Number: D825c
Chemical Formula: $C_{21}H_{32}O_2$
CAS Registry Number: 1235-15-0
Structure:

Description: White solid
Batch Number: 17-S-02
Molecular Weight: 316.5
Release date: 25th September 2017




Synonyms: (17$\alpha$)-(+/-)-13-Ethyl-17-hydroxy-18,19-dinor-pregn-4-en-3-one
*dl*-13$\beta$,17$\alpha$-Diethyl-17$\beta$-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 D825c. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 979 ± 19 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 6890 | |
| | 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 mass fraction of main component : | | |
| | Initial analysis: | Mean = 98.9%, s = 0.01% (7 ampoules in duplicate, October 2017) | |
| | Re-analysis: | Mean = 99.0%, s = 0.02% (5 ampoules in duplicate, October 2018) | |

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

The purity value was obtained from a combination of traditional analytical techniques by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and $^{1}$H NMR spectroscopy. Supporting evidence is provided by HPLC-UV analysis processing at 239 nm, headspace GC-MS analysis of occluded solvents and elemental microanalysis.

| | | | |
|---|---|---|---|
| GC-FID: | Instrument: | Agilent 6890 | |
| | 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 mass fraction of main component : | | |
| | Initial analysis: | Mean = 99.0%, s = 0.04% (10 sub samples in duplicate, July 2017) | |
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler | |
| | Column: | Alltima C-18, 5 μm (4.6 mm x 150 mm) | |
| | Column oven: | 40 °C | |
| | Mobile Phase: | Acetonitrile/MilliQ water (40:60 v/v) | |
| | Flow rate: | 1.0 mL/min | |
| | Detector: | Shimadzu SPD-M20A PDA operating at 239 nm | |
| | Relative mass fraction of main component : | | |
| | Initial analysis: | Mean = 98.95%, s = 0.04% (7 sub samples in duplicate, September 2017) | |
| Thermogravimetric analysis: | | Volatile content 0.2% and non volatile residue < 0.2% mass fraction (August 2017) | |
| Karl Fischer analysis: | | Moisture content 0.1% mass fraction (July 2016) | |

**Spectroscopic and other characterisation data**

GC-MS: Parent compound:

| | | |
|---|---|---|
| Instrument: | Agilent 6890/5973 | |
| Column: | HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm | |
| Program: | 180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min) | |
| Injector: | 250 °C | Transfer line temp: 280 °C |
| Carrier: | Helium, 1.0 mL/min | Split ratio: 20/1 |

*Bis*-TMS derivative:

| | | |
|---|---|---|
| Instrument: | Agilent 6890/5973 | |
| Column: | HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm | |
| Program: | 180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min) | |
| Injector: | 250 °C | Transfer line temp: 280 °C |
| Carrier: | Helium, 1.0 mL/min | Split ratio: 20/1 |

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 mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (12.9 min): 316 ($M^+$, 67), 298 (19), 287 (19), 269 (100), 245 (54), 229 (32), 159 (23), 135 (24), 110 (30), 91 (42), 85 (32), 57 (29) *m/z*

*Bis*-TMS (15.3 min): 460 ($M^+$, 24), 431 (5), 370 (10), 301 (100), 299 (10), 280 (12), 157 (11), 73 (68) *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: | Ethyl acetate, hexane | |

TLC: Conditions: Kieselgel $60F_{254}$. Chloroform/ethyl acetate (4/1)
Single spot observed, $R_f$ = 0.45. Visualisation with UV at 254 nm

IR:

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

$^1$H NMR:

| | | |
|---|---|---|
| Instrument: | Bruker Avance III 500 | |
| Field strength: | 500 MHz | Solvent: $CDCl_3$ (7.26 ppm) |
| Spectral data: | δ 0.80 (1H, m), 0.96 (3H, t, *J* = 7.25 Hz), 1.0 (3H, t, *J* = 7.45 Hz), 1.03-1.63 (13H, m),1.81-1.87 (3H, m), 1.95 (1H, m), 2.10 (1H, m), 2.22-2.32 (3H, m), 2.37-2.44 (1H, m), 2.47 (1H, m), 5.82 (1H, s) ppm | |

Ethyl acetate estimated at 0.8% and hexane estimated at 0.4% mass fraction were observed in the $^1$H NMR.

$^{13}$C NMR:

| | | |
|---|---|---|
| Instrument: | Bruker Avance III 500 | |
| Field strength: | 126 MHz | Solvent: $CDCl_3$ (77.19 ppm) |
| Spectral data: | δ 7.7, 10.1, 20.4, 23.1, 26.6, 26.7, 27.7, 29.9, 31.1, 34.4, 35.7, 36.7, 41.2, 42.7, 47.9, 49.5, 51.2, 85.4, 124.7, 167.0, 200.2 ppm | |

Melting point: 172-175 °C

Microanalysis: Found: C = 79.9%; H = 10.5% (August 2017)
Calc: C = 79.7%; H = 10.2% (Calculated for $C_{21}H_{32}O_2$)

**Expiration of certification**
The property values are valid till 3rd October 2021, 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.

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.

**Metrological traceability**
The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis.

**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**
This reference material should be used for qualitative 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: 26 November, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 26th November 2018.