Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

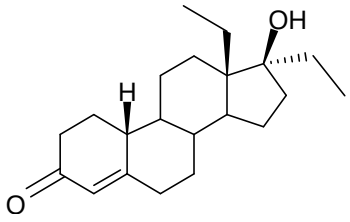
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D825b: Norbolethone

Report ID: D825b.2019.01 (Ampouled 140911)

Chemical Formula: $C_{21}H_{32}O_2$

Molecular Weight: 316.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
06-S-04	**1235-15-0**	**983 ± 33 μg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-13,17-Diethyl-17-hydroxy-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[a]phenanthren-3-one.

Expiration of certification: The property values are valid till 27 August 2024, 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.

Description: 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. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 983 ± 33 μg of anhydrous norbolethone.

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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. The long-term stability of the compound in solution has not been examined.

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.

Safety: Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
4 September 2019

This report supersedes any issued prior to 4 September 2019

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

GC-FID:	Instrument:	Agilent 7890 or Varian CP-3800
	Column:	HP-1MS or VF-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 mass fraction of the main component:	
	Initial analysis:	Mean = 99.1%, s= 0.01% (7 ampoules in duplicate, September 2014)
	Re-analysis:	Mean = 99.1%, s= 0.01% (5 ampoules in duplicate, September 2016)
	Re-analysis:	Mean = 99.2%, s= 0.01% (5 ampoules in duplicate, August 2019)
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 mass fraction of the 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 identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by qualitative elemental microanalysis.

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 mass fraction of the 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 °CDetector Temp: 320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the 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)
	Calculated:	C = 79.7%, H = 10.2% (Calculated for $C_{21}H_{32}O_2$)