



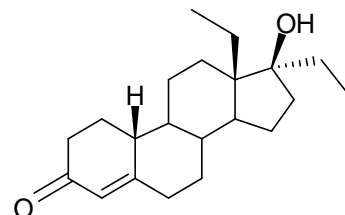
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D825c: Norbolethone

Report ID: D825c.2018.02 (Ampouled 171012)

Chemical Formula: C₂₁H₃₂O₂

Molecular Weight: 316.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
17-S-02	1235-15-0	979 ± 19 µg

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

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

Expiration of certification: The property values are valid till 3 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D825c. 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 979 ± 19 µg of anhydrous norbolethone. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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.

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: In the absence of long term 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 ten years. 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.
5 May 2020

This report supersedes any issued prior to 20 April 2020.

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:

Warning: This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 °C) 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 the 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 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, HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by qualitative 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 the 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 the main component:

Initial analysis: Mean = 98.95%, s = 0.04% (7 sub samples in duplicate, September 2017)

Karl Fischer analysis: Moisture content 0.1% mass fraction (July 2016)

Thermogravimetric analysis: Volatile content 0.2% and non volatile residue < 0.2% mass fraction (August 2017)

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 μ m
	Program:	180 $^{\circ}$ C (1 min), 30 $^{\circ}$ C/min to 250 $^{\circ}$ C (10 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	<i>Bis</i> -TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μ m
	Program:	180 $^{\circ}$ C (1 min), 30 $^{\circ}$ C/min to 250 $^{\circ}$ C (10 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the parent compound and <i>bis</i> -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) <i>m/z</i>
	<i>Bis</i> -TMS (15.3 min):	460 (M^+ , 24), 431 (5), 370 (10), 301 (100), 299 (10), 280 (12), 157 (11), 73 (68) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ 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}
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 $^{\circ}$ C	
Microanalysis:	Found:	C = 79.9%; H = 10.5% (August 2017)
	Calculated:	C = 79.7%; H = 10.2% (Calculated for C ₂₁ H ₃₂ O ₂)