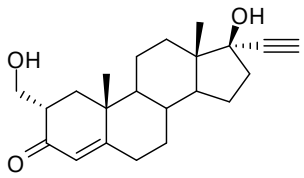
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

Report ID: D920b.2018.01 (Ampouled 181025)
This batch of ampoules was prepared from the bulk material on 25th October 2018.

Compound Name: **2α-Hydroxymethylethisterone**
Collection Number: D920b
Chemical Formula: $C_{22}H_{30}O_3$
CAS Registry Number: 2787-03-3
Structure:



Description: White solid
Batch Number: 17-S-06
Molecular Weight: 342.5
Release date: February 2018

Synonyms: 17β-hydroxy-2α-(hydroxymethyl)pregn-4-en-20-yn-3-one,
17-hydroxy-2α-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one,
17α-ethynyl-2α-hydroxymethyl-4-androsten-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 D920b. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. Acetonitrile). This will transfer 994 ± 16 μg of anhydrous 2α-hydroxymethylethisterone. The uncertainty is stated at the 95% coverage interval.

HPLC:
Column: Alltima C-18, 5 μm (4.6 mm × 150 mm)
Mobile Phase: A = MilliQ water; B = Acetonitrile
0-17 min 35% B; 17-20 min 35-50% B; 20-25 min 50% B; 25-26 min 50-35% B, 26-35 min 35% B
Flow Rate: 1.0 mL/min
Detector: PDA at 247 nm
Relative peak area of main component:
Initial analysis: Mean = 98.8%, s = 0.01% (7 ampoules in duplicate, November 2018)

The purity estimate by HPLC-UV analysis is a measure of 2α-hydroxymethylethisterone and 2β-hydroxymethylethisterone combined.

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

The stated purity, obtained by subtraction from 100% of total impurities by HPLC with UV detection, Karl Fischer, thermogravimetric analysis and ^{1}H NMR spectroscopy, represents a combination of 2α-hydroxymethylethisterone and the 2β-hydroxymethyl epimer (ca. 3.7% mass fraction). This material is also contaminated with a dihydro derivative presumed to be 17α-ethenyl-2α-hydroxymethyl-4-androsten-3-one (ca. 1.0% mass fraction). Other impurities have been observed but not identified, nor accurately quantified. This material is recommended for qualitative analysis, the purity value being indicative only.

HPLC:	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-17 min 35% B; 17-20 min 35-50% B; 20-25 min 50% B; 25-26 min 50-35% B, 26-35 min 35% B
	Flow Rate:	1.0 mL/min
	Detector:	PDA at 247 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 98.7%, s = 0.04% (10 sub samples in duplicate, February 2018)
	The purity estimate by HPLC-UV analysis is a measure of 2α-hydroxymethylethisterone and 2β-hydroxymethylethisterone combined.	
Thermogravimetric analysis:		Volatile content <0.1% and non volatile residue < 0.2% mass fraction (February 2018)
Karl Fischer analysis:		Moisture content 0.2% mass fraction (January 2018)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973	
	Column:	Ultra 1, 17 m x 0.2 mm I.D.x 0.11 μm	
	Program:	189 °C (0.2 min) 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, 1.0 mL/min	Split ratio: 14/1

The retention time of the *tris*-TMS derivative is reported with the major peaks in the mass spectra. The latter is reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

18.1 min: 558 (M^+, 46), 543 (4), 455 (4), 403 (2), 193 (8), 147 (14), 103 (11), 73 (100) *m/z*

Note: Both 2α-hydroxymethylethisterone and 2β-hydroxymethylethisterone form the same *tris*-TMS compound when derivatisation conditions result in formation of the 3-O-TMS group.

LC-MS:	Instrument	Micromass Quatro Micro
	Operation:	Positive ion mode, buffer/water/acetonitrile gradient, flow at 0.2mL/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	34 V
	Major peak:	8.7 min, 343 ($M+H^+$) *m/z*
	Minor peak	9.1 min, 345 ($M+H^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate / chloroform (1/1). Single spot observed, R_f = 0.28. Visualisation with UV at 254 nm.
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3393, 2942, 2103, 1672, 1620, 1447, 1375, 1215, 1048, 995, 862, 637 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.88 (3H, s), 0.96 (1H, m), 1.05 (1H, m), 1.23 (3H, s), 1.30-1.75 (9H, m), 1.85 (1H, m), 1.90 (1H, dd, *J* = 4.7, 13.1 Hz), 1.99 (1H, ddd, *J* = 3.8, 13.7, 13.7 Hz), 2.17 (1H, s), 2.25-2.32 (2H, m), 2.37 (1H, ddd, J = 4.3, 13.8, 13.8 Hz), 2.55 (1H, s), 2.57 (1H, m), 3.24 (1H, dd, *J* = 4.1, 9.0 Hz), 3.68 (1H, m), 3.75 (1H, m), 5.72 (1H, s) ppm
		Methanol estimated at 0.1% mass fraction was observed in the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	151 MHz Solvent: $CDCl_3$ (77.2ppm)
	Spectral data:	δ 12.7, 17.7, 20.6, 23.0, 31.3, 32.3, 32.5, 36.0, 38.7, 38.8, 39.2, 43.6, 46.6, 49.8, 53.8, 63.8, 74.1, 79.5, 87.2, 123.5, 171.9, 202.7 ppm
Melting point:		156-159 °C
Microanalysis:		Found: C = 77.1 %, H = 9.1 % (February 2018) Calc: C = 77.2 %, H = 8.8 % (Calculated for $C_{22}H_{30}O_3$)

Expiration of certification
The property values are valid till 1st November 2021, i.e. three years from the date of 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 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 HPLC with UV detection 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
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: 7 December, 2018.