Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

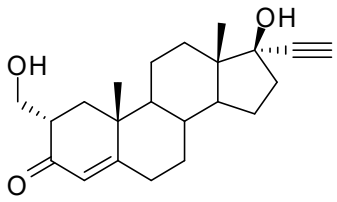
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D920b: 2α-Hydroxymethylethisterone

Report ID: D920b.2024.01 (Ampouled 181025)

Chemical Formula: $C_{22}H_{30}O_3$

Molecular Weight: 342.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
17-S-06	2787-03-3	991 ± 6 μg

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

IUPAC name: (2α,17ξ)-17-Hydroxy-2-(hydroxymethyl)pregn-4-en-20-yn-3-one

Expiration of certification: The property values are valid till 11 July 2027, 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 and is intended for a single use to prepare a standard solution containing D920b. 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. acetonitrile). This will transfer 994 ± 6 μg of anhydrous 2α-hydroxymethylethisterone.

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 three 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 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.

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.
6 August 2024

This report supersedes any issued prior to 6 August 2024.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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

Characterisation Report:

HPLC:	Instrument:	Waters Alliance 2650 or Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = Milli-Q 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:	Waters 2998 or Shimadzu SPD-M20A PDA operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.8%, s = 0.01% (7 ampoules in duplicate, November 2018)
	Re-analysis:	Mean = 98.8%, s = 0.01% (5 ampoules in duplicate, January 2020)
	Re-analysis:	Mean = 98.8%, s = 0.003% (5 ampoules in duplicate, September 2022)
	Re-analysis:	Mean = 98.5%, s = 0.005% (5 ampoules in duplicate, July 2024)
	The purity estimate by HPLC-UV analysis is a measure of 2α-hydroxymethylethisterone and 2β-hydroxymethylethisterone combined.	

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 HPLC with UV detection, 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 elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	A = Milli-Q 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:	Shimadzu SPD-M20A PDA operating at 247 nm
	Relative mass fraction of the 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 0.2 mL/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 = 0.3. Visualisation with UV at 254 nm.
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3393, 3286, 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:	$CDCl_3$ (77.2 ppm)
	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)
	Calculated:	C = 77.2 %, H = 8.8 % (Calculated for $C_{22}H_{30}O_3$)