



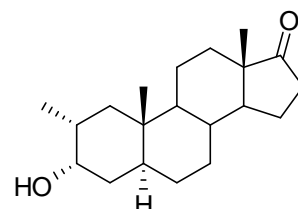
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D567: 2 α -Methyl-5 α -androstan-3 α -ol-17-one

Report ID: D567.2024.01 (Ampouled 120403)

Chemical Formula: C₂₀H₃₂O₂

Molecular Weight: 304.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-S-01	6961-54-2	993 ± 15 µg

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

IUPAC name: (2R,3S,5S,8R,9S,10S,13S,14S)-3-hydroxy-2,10,13-trimethyl-1,2,3,4,5,6,7,8,9,11,12,14,15,16-tetradecahydrocyclopenta[a]phenanthren-17-one

Expiration of certification: The property values are valid till 15 November 2029, 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 sample bottles 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 D567. This material was prepared by synthesis or sourced from an external supplier, certified for identity and purity by NMI Australia.

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 993 ± 15 µg of anhydrous 2 α -methyl-5 α -androstan-3 α -ol-17-one. 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 all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of 7 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.
21 November 2024

This report supersedes any issued prior to 21 November 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:

GC-FID:	Instrument:	Agilent 7890N or 8890	
	Column:	HP-1MS, 30 m \times 0.32 mm I.D. \times 0.25 μ m	
	Program:	190 $^{\circ}$ C (0.5 min), 6 $^{\circ}$ C/min to 220 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)	
	Injector:	250 $^{\circ}$ C	Detector Temp: 320 $^{\circ}$ C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.0%, s = 0.002% (7 ampoules in duplicate, April 2012)	
	Re-analysis:	Mean = 99.0%, s = 0.001% (5 ampoules in duplicate, March 2013)	
	Re-analysis:	Mean = 98.8%, s = 0.15% (5 ampoules in duplicate, February 2016)	
	Re-analysis:	Mean = 99.0%, s = 0.005% (5 ampoules in duplicate, January 2019)	
	Re-analysis:	Mean = 99.1%, s = 0.007% (5 ampoules in duplicate, November 2024)	

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, and Karl Fischer analysis. 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 ^1H NMR spectroscopy and elemental microanalysis.

GC-FID:	Instrument:	HP5890/Agilent 6890/Agilent 7890	
	Column:	HP-1MS Capillary, 30 m \times 0.32 mm I.D. \times 0.25 μ m	
	Program:	190 $^{\circ}$ C (0.5 min), 6 $^{\circ}$ C/min to 220 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)	
	Injector:	250 $^{\circ}$ C	Detector Temp: 320 $^{\circ}$ C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.46%, s = 0.27% (7 sub samples in duplicate, March 1999)	
	Re-analysis:	Mean = 98.75%, s = 0.14% (8 sub samples in duplicate, September 2006)	
	Re-analysis:	Mean = 98.98%, s = 0.15% (5 sub samples in duplicate, April 2012)	

HPLC:	Peak area percentage of total: > 99% (3 samples)		
	Column:	Alltima C-18, 5 μ m (4.6 mm \times 150 mm)	
	Mobile Phase:	Acetonitrile/water (65:35)	
	Flow Rate:	1 mL/min	
	Detector:	Refractive index	

Karl Fischer analysis: Moisture content \leq 0.1% mass fraction (April 2012)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.3% mass fraction (September 2006)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 0.10 μ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 220 $^{\circ}$ C, 20 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	280 $^{\circ}$ C, Split injection
	Transfer line temp:	300 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 <i>m/z</i>
	<i>Bis</i> -TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μ m
	Program:	170 $^{\circ}$ C (0.5 min), 3 $^{\circ}$ C/min to 234 $^{\circ}$ C, 10 $^{\circ}$ C/min to 265 $^{\circ}$ C (2 min)
	Injector:	280 $^{\circ}$ C, Split injection
	Transfer line temp:	300 $^{\circ}$ C
	Carrier:	Helium
	Scan range:	50-550 <i>m/z</i>
	The retention times of the parent compound and <i>bis</i> -TMS derivative are reported 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 (5.9 min):	304 (M^+ , 100), 271 (34), 260 (26), 121 (66), 108 (44), 93 (36) <i>m/z</i>
	<i>Bis</i> -TMS (9.7 min):	448 (M^+ , 52), 433 (64), 343 (29), 253 (11), 169 (25), 73 (100) <i>m/z</i>
	The <i>bis</i> -silylated derivative of the synthetic material co-elutes on GC-MS with a comparison sample of 2 α -methyl-5 α -androstan-3 α -ol-17-one. The two materials give matching mass spectra.	
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate/chloroform (30:10:5) Single spot observed, R_f = 0.2 (5 samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1} , KBr pellet
	Peaks:	3472, 1727, 1451, 1403, 1369, 1006, 989 cm^{-1}
¹ H NMR:	Instrument:	Bruker Advance - 300
	Field strength:	300 MHz
	Solvent:	CDCl ₃ (7.26 ppm)
	Key spectral data:	δ 0.82 (3H, s), 0.86 (3H, s), 0.93 (3H, d), 3.77 (1H, br s) ppm
¹³ C NMR	Instrument:	Bruker Advance - 300
	Field strength:	75 MHz
	Solvent:	CDCl ₃ (77.16 ppm)
	Spectral data:	δ 12.5, 14.2, 18.8, 20.4, 22.1, 28.3, 31.2, 31.9, 32.1, 35.3, 36.2, 36.9, 39.1, 41.1, 48.2, 51.9, 54.9, 70.9, 221.7 ppm
X-ray crystallography:	Relative stereochemical configuration confirmed, R = 6.6%	
Melting point:	202 – 204 $^{\circ}$ C	
Microanalysis:	Found:	C = 79.0%; H = 10.3% (March 1999)
	Calculated:	C = 78.9%; H = 10.6% (Calculated for C ₂₀ H ₃₂ O ₂)