Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

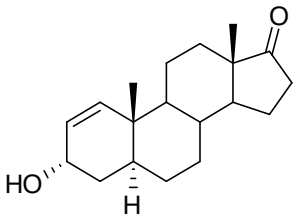
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D832: 5α-Androst-1-en-3α-ol-17-one

Report ID: D832.2021.01 (Ampouled 151013)

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
03-S-06	38859-37-9	~200 μg

IUPAC name: (3α,5α)-3-Hydroxyandrost-1-en-17-one.

Expiration of certification: The property values are valid till 8 July 2024, 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 RM is intended for a single use to prepare a standard solution containing D832. This material was prepared by synthesis and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

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 approximately 200 μg of anhydrous 5α-androst-1-en-3α-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 ºC in a closed container in a dry, dark area.

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.
14 July 2021

This report supersedes any issued prior to 14 July 2021.

Characterisation Report:

GC-FID	Instrument:	Varian CP-3800 or Agilent 7890A
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 µm
	Program	140 °C (1 min), 30 °C /min to 230 °C (13 min), 30 °C /min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area response of the main component:	
	Initial analysis:	Mean = 83.8%, s = 0.13% (7 ampoules in duplicate, October 2015)
	Re-analysis:	Mean = 84.0%, s = 0.09% (5 ampoules in duplicate, September 2016)
	Re-analysis:	Mean = 83.6%, s = 0.07% (5 ampoules in duplicate, August 2017)
	Re-analysis:	Mean = 84.4%, s = 0.07% (5 ampoules in duplicate, September 2018)
	Re-analysis:	Mean = 83.6%, s = 0.06% (5 ampoules in duplicate, July 2021)

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 elemental microanalysis.

GC-FID	Data derived from injections of native D832 and *bis*-trimethylsilyl derivatives:	
	Instrument:	Agilent6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program	140 °C (1 min), 40 °C /min to 190 °C (20 min), 40 °C /min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area response of the main component:	
	Initial analysis:	Mean = 84.8%, s = 1.0% (5 samples in duplicate, March 2004)
Thermogravimetric analysis:		Volatiles content 1.3% and non-volatile residue 0.3% mass fraction (March 2004 & August 2005)

Spectroscopic and other characterisation data

GC-MS: *Bis*-trimethylsilyl derivative:

Instrument:	Agilent 6890/5973
Column:	Ultra 1, 17 m × 0.2 mm I.D. × 0.11 μm
Program:	189 °C (0.2 min) 3 °C /min to 240 °C, 10 °C /min to 265, 30 °C/min to 310 °C
Injector:	250 °C
Transfer line temp:	300 °C
Carrier:	Helium 1.0 mL/min
Split ratio:	14/1

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

Bis-TMS (9.5 min): 432 (M^+, 62), 417 (63), 327 (20), 290 (24), 275 (41), 169 (23), 73 (100) *m/z*

IR:

Instrument:	FT-IR, Biorad FTS3000MX
Range:	4000-400 cm^{-1}, KBr
Peaks:	3530, 3025, 2927, 1734, 1454, 1376, 1260, 1053, 1005, 750 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.83 (3H, s), 0.88 (3H, s), 4.11 (1H, t, *J* = 4.6 Hz), 5.66 (1H, m), 6.06 (1H, d, *J* = 10.0 Hz) ppm

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	126 MHz
Solvent:	$CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.8, 13.9, 20.4, 21.8, 27.7, 30.8, 31.5, 34.7, 35.3, 35.8, 38.1, 39.0, 47.9, 51.1, 51.5, 64.3, 126.3, 139.9, 221.1 ppm