Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

CIPM MRA

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

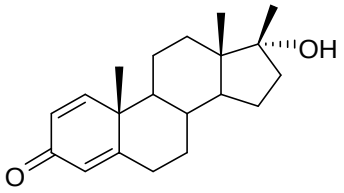
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D562: 17-Epimethandienone

Report ID: D562.2016.03 (Ampouled 070319)

Chemical Formula: $C_{20}H_{28}O_2$

Molecular Weight: 300.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-000007	**NA**	**1000 ± 26 μg**

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

IUPAC name: (17α)-17-Hydroxy-17-methylandrosta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 7 December 2021, i.e. 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 and is intended for a single use to prepare a standard solution containing D562. 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 1000 ± 26 μg of anhydrous 17-epimethandienone.

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 five 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 1-2 mg sub samples 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 a 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.
20 December 2019

This report supersedes any issued prior to 20 December 2019

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.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see http://www.bipm.org).

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 6890N, HP5890 or Varian CP-3800
	Columns:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
		VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm
		ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.04% (7 ampoules in duplicate, November 2007)
	Re-analysis:	Mean = 99.7%, s = 0.03% (5 ampoules in duplicate, February 2008)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, March 2009)
	Re-analysis:	Mean = 99.6%, s = 0.1% (5 ampoules in duplicate, February 2012)
	Re-analysis:	Mean = 99.3%, s = 0.2% (5 ampoules in duplicate, December 2016)

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 ^{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:	Instrument:	HP5890
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.05% (10 sub samples in duplicate, December 1998)
	Re-analysis:	Mean = 99.6%, s = 0.02% (10 sub samples in duplicate, June 2006)
HPLC:	Column:	Spherisorb ODSII, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (50:50)
	Flow Rate:	1.0 mL/min
	Detector:	UV detection at 254 nm
	Retention time:	21.1 min
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8% (5 sub samples)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (February 1999 and June 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 μm
Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)
Injector:	280 °C Split inj.
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min
Scan range:	50-550 *m/z*

Bis-TMS derivative:

Instrument:	HP 6890/5973
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm
Program:	170 °C (1 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
Injector:	280 °C Split inj.
Transfer line temp:	300 °C
Carrier:	Helium
Scan range:	50-550 m/z

The retention times of the parent material and its *bis*–TMS derivative are reported along with the major peaks in the mass spectra. The latter are reported as mass to charge ratios and (in brackets) as a percentage relative to the intensity of the base peak.

Parent (6.7 min):	300 (M^+, 1), 282 (31), 267 (19), 161 (45), 122 (100), 121 (70) *m/z*
Bis –TMS (10.9 min):	444 (M^+, 45), 339 (58), 229 (10), 206 (100), 73 (87) *m/z*

The *bis*-silylated derivative of the synthetic material co-elutes on GC-MS with a derivatised comparison sample of 17-epimethandienone and the two materials produce matching mass spectra.

TLC:

Conditions:	Kieselgel 60F_{254}. Ethyl acetate/hexane (50:50) Single spot observed, R_f = 0.36 (3 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3487, 1666, 1622, 1600, 1377, 886 cm^{-1}

^{1}H NMR:

Instrument:	Bruker ARX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.75 (3H, s), 1.20 (3H, s), 1.24 (3H, s), 6.06 (1H, t), 6.22 (1H, dd), 7.07 (1H, d) ppm

^{13}C NMR:

Instrument:	Bruker ARX-500
Field strength:	125 MHz
Solvent:	$CDCl_3$ (77.16 ppm)
Spectral data:	δ 15.9, 18.7, 22.4, 22.7, 24.0, 29.6, 32.9, 33.7, 36.0, 38.3, 43.6, 46.7, 48.9, 52.2, 81.8, 123.8, 127.4, 155.9, 169.3, 186.4 ppm

Melting point: 220-221 °C

Microanalysis:

Found:	C = 80.0%; H = 9.5%
Calculated:	C = 80.0%; H = 9.4% (Calculated for $C_{20}H_{28}O_2$)