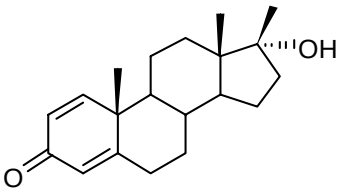
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Compound Name: **17-Epimethandienone**
Collection No: D562
Chemical Formula: $C_{20}H_{28}O_2$
CAS Number: 33526-40-8
Structure:



Description: White crystals
Batch No: 99-000007
Molecular Weight: 300.4
Batch production completed: December 1998
Metabolite of methandienone

Synonym: 17α-Hydroxy-17β-methylandrosta-1,4-dien-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 D562. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform). This will transfer 1.000 ± 0.026 mg of anhydrous 17-epimethandienone.

Warning: This material is sensitive to the quality of the GC silanised glass injection liner.

GC-FID:

Instrument:	HP5890	
Column:	ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm	
Program:	180 °C (1 min), 15 °C/min to 300 °C (3 mins).	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area response of 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 (7 sub samples in duplicate, June 2006)
Re-analysis:	Mean = 99.6%, s = 0.04 (7 ampoules in duplicate, March 2007)
Current re-analysis	Mean = 99.7%, s = 0.03 (5 ampoules in duplicate, February 2008)

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

Purity estimate obtained by subtraction from 100% of total impurities by GC-FID, TGA and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

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, hold.	
Injector:	280 °C Split inj.	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min.	Scan range: 50-550 a.m.u.

Bis-TMS derivative:

Instrument:	HP 6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm	
Program:	170 °C, rate rise 3 °C/min to 234 °C, 10 °C/min to 265 °C, hold.	
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 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.

HPLC:	Peak area percentage of total > 99.8% (5 samples)	
	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 Detention time: 21.1 min
TLC:	Conditions:	Kieselgel $60F_{254}$. Ethyl acetate/hexane (50:50) Single spot observed, $R_f = 0.36$ (3 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: $CDCl_3$
	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: $CDCl_3$
	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% Calc: C = 80.0%, H = 9.4%
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction. (February 1999 and June 2006)

Expiration of certification:	The certified values are valid till 20th February 2009, i.e. one year from the date of re-certification, provided the 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 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 5 randomly selected 1 mg ampoules of the material. The material was judged to be homogenous 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:	For ***in vitro*** laboratory 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: 6 March, 2008
Report ID:	D562.2008.01 (Ampouled 070319) Characterisation data and certified property values specified in this report supersede those in all reports issued prior to 7th March 2008.

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