



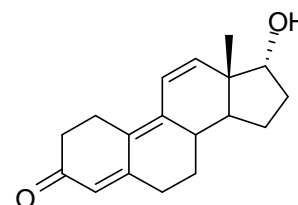
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D708: 17 α -Trenbolone

Report ID: D708.2019.01 (Bottled 190620)

Chemical Formula: C₁₈H₂₂O₂

Molecular Weight: 270.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
01-AV-09	80657-17-6	98.5 \pm 0.8%

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

IUPAC name: (17 α)-17-Hydroxyestra-4,9,11-trien-3-one. (Metabolite of trenbolone)

Expiration of certification: The property values are valid till 22 July 2024, 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: Light yellow powder sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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 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 five 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.
7 August 2019

This report supersedes any issued prior to 7 August 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.

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

Characterisation Report:

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 ¹H NMR spectroscopy. 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 elemental microanalysis.

STABILITY WARNING: Solutions prepared from this material are not stable when exposed to air or light. Where possible they should be used immediately. If this is not convenient, store solutions out of direct light at or below 4 °C and monitor regularly for possible decomposition.

GC-FID: Instrument: Agilent 6890N
 Column: HP-1 Capillary, 29.30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 °C (1 min), 15 °C/min to 250 °C (3min), 30 °C/min to 310 °C (5min)
 Injector: 250 °C Detector Temp: 320 °C
 Carrier: Helium Split ratio: 20/1
 Relative mass fraction of the main component:
 Initial analysis: Mean = 99.2%, s = 0.08% (10 sub samples in duplicate, September 2001)
 Re-analysis: Mean = 98.9%, s = 0.02% (5 sub samples in duplicate, June 2008)

GC-FID: Instrument: Agilent 6890N
 Column: HP-1 Capillary, 29.30 m \times 0.32 mm I.D. \times 0.25 μ m
 Program: 180 °C (1 min), 15 °C/min to 250 °C (3min), 30 °C/min to 310 °C (5 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 = 0.1% (5 sub samples in duplicate, June 2011)
 Re-analysis: Mean = 98.8%, s = 0.08% (5 sub samples in duplicate, March 2016)

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
 Column: Alltima C18, 5 μ m (4.6 mm \times 150 mm)
 Column oven: 55 °C
 Mobile Phase: Methanol/MilliQ water (60:40 v/v)
 Flow rate: 1.0 mL/min
 Detector: Waters 2998 PDA operating at 340 nm
 Relative peak area of the main component:
 Initial analysis: Mean = 99.8%, s = 0.01% (3 sub samples in duplicate, October 2001)

Karl Fischer analysis: Moisture content 0.3% mass fraction (May and June 2008)
 Moisture content 0.2% mass fraction (June 2011)
 Moisture content 0.2% mass fraction (July 2019)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (November 2001 and June 2006)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP 5890/5973
	Column:	HP5-MS, 12 m \times 0.25 mm I.D. \times 0.11 μ m
	Program:	150 $^{\circ}$ C (2.0 min), 10 $^{\circ}$ C /min to 280 $^{\circ}$ C
	Injector:	180 $^{\circ}$ C
	Split ratio:	NA
	Transfer line temp:	300 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 <i>m/z</i>
	The retention time of the parent compound is reported 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.	
	Parent (11.6 min):	270 (M^+ , 100), 252 (99), 237 (51), 155 (69), 141 (79), 128 (76), 115 (68) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate (2:3) Single spot observed, $R_f = 0.3$
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1} , KBr disk
	Peaks:	3425, 2942, 1639, 1569, 1278, 1233, 1087, 1029, 862, 765 cm^{-1}
¹ H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	CDCl ₃ (7.26 ppm)
	Spectral data:	δ 0.79 (3H, s), 1.35 (2H, m), 1.58 (1H, ddd), 2.43 (2H, t), 3.95 (1H, d), 5.75 (1H, s), 6.38 (1H, d), 6.54 (1H, d) ppm Ethyl acetate estimated at 0.2% mass fraction was observed in the ¹ H NMR
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	CDCl ₃ (77.0 ppm)
	Spectral data:	δ 18.5, 23.5, 24.2, 27.6, 31.5, 32.9, 36.6, 37.6, 46.1, 48.4, 78.0, 123.5, 125.4, 127.1, 141.3, 142.1, 156.7, 199.3 ppm
Melting point:		106-109 $^{\circ}$ C
Microanalysis:	Found:	C = 80.1%; H = 8.4% (July, 2001)
	Calculated:	C = 80.0%; H = 8.2% (Calculated for C ₁₈ H ₂₂ O ₂)