Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

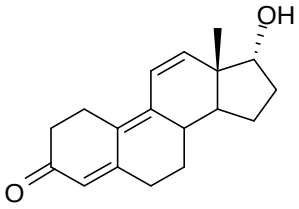
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D708b: 17α -Trenbolone

Report ID: D708b.2023.01 (Ampouled 230907)

Chemical Formula: $C_{18}H_{22}O_2$

Molecular Weight: 270.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
23-S-04	80657-17-6	557 ± 63 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-Hydroxyestra-4,9,11-trien-3-one. (Metabolite of trenbolone)

Expiration of certification: The property values are valid till 22 November 2026, three years from the date of 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

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 D708b. 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. methanol). This will transfer 557 ± 63 μg of 17α-trenbolone.

Recommended storage: When not in use, this material should be stored at or below -18 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.

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 short term accelerated 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 HPLC with UV detection 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.
28 November 2023

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:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified mass of 17α-trenbolone per ampoule was determined by HPLC analysis against an NMI Australia certified internal standard of trenbolone acetate S024.

QHPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v)
	Flow rate:	1.0 mL/min
	Detector:	RS Diode Array Detector operating at 340 nm
	Internal standard:	Trenbolone acetate (95.3% mass fraction)
	Initial analysis:	Mass per ampoule (557μg), s = 10 μg (7 sub samples, September 2023)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 8890/5977B
	Column:	HP-5MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	160 °C (1 min), 10 °C/min to 220 °C (7 min), 20 °C/min to 300 °C (7 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	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 (18.6 min):	270 (M^+, 26), 252 (28), 237 (16), 207 (100), 181 (31), 165 (35), 155 (30), 141 (36), 128 (37), 115 (33) *m/z*
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:	MeOH-*d*$_4$ (3.31 ppm)
	Spectral data:	δ 0.82 (3H, s), 1.38 (2H, m), 1.57 (1H, ddd), 1.84 (1H, m), 1.91-2.01 (2H, m), 2.20-2.27 (2H, m), 2.45 (2H, t, *J* = 7.3 Hz), 2.57-2.67 (2H, m), 2.78-2.92 (2H, m), 3.90 (1H, d, *J* = 6.0 Hz), 5.76 (1H, s), 6.48 (1H, d, *J* = 10.0 Hz), 6.59 (1H, d, *J* = 10.0 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH-*d*$_4$ (49.0 ppm)
	Spectral data:	δ 18.9, 24.6, 25.2, 29.0, 32.7, 33.3, 37.5, 39.2, 47.5, 79.0, 123.5, 125.6, 127.8, 144.3, 145.0, 160.2, 202.1 ppm
	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