



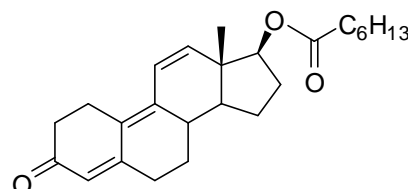
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S028: Trenbolone enanthate

Report ID: S028.2021.01 (Bottled 160822)

Chemical Formula: C₂₅H₃₄O₃

Molecular Weight: 382.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
14-S-05	1629618-98-9	91.8 ± 1.8%

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

IUPAC name: (17β)-3-Oxoestra-4,9,11-trien-17-yl heptanoate

Expiration of certification: The property values are valid till 12 August 2026, 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 solid 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%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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 HPLC with UV detection on ten 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.
30 August 2021

This report supersedes any issued prior to 30 August 2021.

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 14214. 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 quantitative nuclear magnetic resonance (qNMR). A combination of the one-proton doublet of doublets at 4.81 ppm, and the one-proton singlet at 5.78 ppm were measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC: Instrument: Shimadzu or Waters Binary pump
 Column: Alltima C-18, 5 µm (4.6 mm x 150 mm)
 Column oven: 40 °C
 Mobile Phase: Methanol/MilliQ water (90:10 v/v)
 Flow rate: 1.0 mL/min
 Detector: Shimadzu SPD-M20A PDA or Waters 2998 PDA operating at 340 nm
 Relative peak area of the main component:
 Initial analysis: Mean = 96.8%, s = 0.02% (10 sub samples in duplicate, June 2014)
 Re-analysis: Mean = 98.0%, s = 0.04% (5 sub samples in duplicate, July 2015)
 Re-analysis: Mean = 97.9%, s = 0.03% (5 sub samples in duplicate, June 2016)
 Re-analysis: Mean = 98.1%, s = 0.02% (5 sub samples in duplicate, July 2017)
 Re-analysis: Mean = 98.1%, s = 0.02% (5 sub samples in duplicate, September 2018)
 Re-analysis: Mean = 98.1%, s = 0.04% (5 sub samples in duplicate, August 2021)

Karl Fischer analysis: Moisture content ≤ 0.1% mass fraction (June 2014, 2015 and 2016)
 Moisture content 0.2% mass fraction (May 2017)
 Moisture content 0.2% mass fraction (May 2018)
 Moisture content ≤ 0.1% mass fraction (August 2021)

Thermogravimetric analysis: Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (June 2014)

QNMR: Instrument: Bruker Avance-III-500
 Field strength: 500 MHz
 Solvent: CDCl₃ (7.26 ppm)
 Internal standard: Dimethyl terephthalate (100.0% mass fraction)
 Initial analysis: Mean (4.81 ppm) = 91.8%, s = 0.2% (3 sub samples, July 2014)
 Initial analysis: Mean (5.78 ppm) = 91.8%, s = 0.4% (3 sub samples, July 2014)

Spectroscopic and other characterisation data

GC-MS: Instrument: HP6890/5973
 Column: TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μ m
 Program: 250 °C (1 min), 30 °C/min to 300 °C (10 min)
 Injector: 250 °C Split ratio: 20/1
 Transfer line temp: 280 °C Carrier: Helium
 Scan range: 50-550 *m/z*

The retention time of the parent compound is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.4 min): 382 (M^+ , 16), 270 (38), 252 (100), 214 (18), 213 (17), 141 (9), 113 (12), 85 (8), 55 (8) *m/z*

LC-MS: Instrument: Waters 2695 (HPLC)/Micromass Quatro
 Column: Alltima C-18, 5 μ m (4.6 mm x 150 mm)
 Column temp: 40 °C
 Solvent system: 2% Formic acid in water [1%], methanol [90% v/v], water [9% v/v]
 Flow rate: 0.2 mL/min
 Sample prep: 50 μ g/g in MeOH/MilliQ water (25:75)
 Injection volume: 10 μ L
 Ionisation mode: Electrospray positive ion
 Capillary voltage: 3.5 kV Cone voltage: 8 V
 Source temp: 130 °C Desolvation gas temp: 350 °C
 Cone gas flow rate: 27 L/hr Desolvation gas flow: 745 L/hr

The retention time of trenbolone enanthate is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.

5.9 min: 383 ($M+H^+$) *m/z*

HS-GC-MS: Instrument: Agilent 6890/5973/G1888
 Column: DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
 Program: 50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
 Injector: 150 °C
 Transfer line temp: 280 °C
 Carrier: Helium, 1.2 mL/min
 Split ratio: 50/1
 Solvents detected: Hexane

TLC: Conditions: Kieselgel 60F₂₅₄. Hexane/acetone (1:1)
 Single spot observed, R_f = 0.8. Visualisation with UV at 254 nm.

IR: Instrument: Biorad FTS3000MX FT-IR
 Range: 4000-400 cm^{-1} , KBr powder
 Peaks: 2951, 2932, 2858, 1731, 1654, 1585, 1572, 1377, 1238, 1172, 1100, 1016, 993, 888, 785, 773, 686 cm^{-1}

¹H NMR: Instrument: Bruker Avance III-500
 Field strength: 500 MHz
 Solvent: CDCl₃ (7.26 ppm)
 Spectral data: δ 0.89 (3H, t, J = 6.9 Hz), 0.94 (3H, s), 1.25-1.36 (6H, m), 1.44-1.77 (7H, m), 1.89 (1H, m), 2.27 (1H, m), 2.33 (2H, t, J = 7.5 Hz), 2.39-2.48 (3H, m), 2.50-2.62 (2H, m), 2.81 (2H, m), 4.81 (1H, dd, J = 7.4, 9.3 Hz), 5.78 (1H, s), 6.36 (1H, d, J = 9.9 Hz), 6.43 (1H, d, J = 9.9 Hz) ppm

¹³C NMR: Instrument: Bruker Avance III-500
 Field strength: 126 MHz
 Solvent: CDCl₃ (77.2 ppm)
 Spectral data: δ 14.2, 14.6, 22.7, 23.3, 24.5, 25.2, 27.1, 27.7, 29.0, 31.57, 31.62, 34.7, 36.8, 37.7, 45.2, 47.8, 78.3, 123.8, 124.0, 127.5, 141.6, 141.9, 156.4, 174.1, 199.4 ppm

Melting point: 73-75 °C

Microanalysis: Found: C = 77.4%; H = 8.8% (June, 2014)
 Calculated: C = 78.5%; H = 9.0% (Calculated for C₂₅H₃₄O₃)