



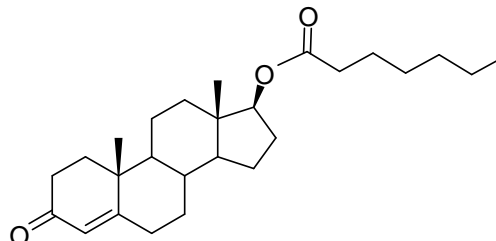
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D773d: Testosterone enanthate

Report ID: D773d.2025.01

Chemical Formula:  $C_{26}H_{40}O_3$

Molecular Weight: 400.6 g/mol



### Certified value

Batch No.	CAS No.	Purity (mass fraction)
20-S-03	315-37-7	99.2 ± 3.8%

**IUPAC name:** (17 $\beta$ )-3-Oxoandrost-4-en-17-yl heptanoate.

**Expiration of certification:** The property values are valid till 21 May 2030, 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 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. 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:** White solid prepared by synthesis and certified for identity and purity by NMI Australia. Packaged in amber glass bottles with a septum and crimped aluminium 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 -18 °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:** Previous batches of testosterone enanthate have been shown to decompose over time to a hydroxylated species. The rate of decomposition has been shown to increase with time and will be monitored on an three-year basis. 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.  
11 June 2025

This report supersedes any issued prior to 11 June 2025.

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.

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## Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis, and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

$I_{\text{ORG}}$  = Organic impurities of related structure,  $I_{\text{VOL}}$  = volatile impurities,  $I_{\text{NVR}}$  = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Grace Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile / Milli-Q water (85:15 v/v)
	Flow rate:	1.2 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 240 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (10 sub samples in duplicate, June 2020)
	Re-analysis:	Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, March 2021)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, November 2021)
	Re-analysis:	Mean = 99.8%, s = 0.01% (6 sub samples in duplicate, October 2022)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, July 2023)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, May 2025)

Karl Fischer analysis:	Moisture content 0.2% mass fraction (June 2020)
	Moisture content < 0.1% mass fraction (March 2021, November 2021, October 2022, and July 2023)

Thermogravimetric analysis:	The volatile content (e.g. organic solvents and/or water) could not be determined because of the inherent volatility of the material and non-volatile residue < 0.2% mass fraction (June 2020).
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## Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	DB-5MS, 30 m x 0.25 mm I.D. x 0.25 µm
	Program:	200 °C (1 min), 15 °C/min to 260 °C (5 min), 30 °C/min to 300 °C (10 min)
	Injector:	250 °C,
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-700 <i>m/z</i>
	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 (16.8 min):	400 ( <i>M</i> <sup>+</sup> , 24), 358 (19), 288 (20), 270 (15), 245 (9), 228 (34), 213 (10), 185 (18), 147 (46), 131 (14), 124 (100), 113 (69), 107 (20), 93 (25), 55 (32), 43 (72) <i>m/z</i>
LC-MS:	Instrument:	Waters Alliance/ Micromass Quattro TQ Detector
	Column:	X-Bridge C-18, 100 mm x 2.1 mm I.D. x 3.5 µm
	Column temp:	40 °C
	Solvent system:	2 percent formic acid [5% v/v], acetonitrile [85% v/v], MilliQ water [10% v/v]
	Flow rate:	0.2 mL/min
	Sample prep:	1000 µg/g in acetonitrile
	Injection volume:	10 µL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	3.5 kV
	Cone voltage:	15 V
	Source temp:	130 °C
	Desolvation gas temp:	350 °C
	Cone gas flow rate:	27 L/hr
	Desolvation gas flow:	764 L/hr
	The retention time of testosterone enanthate is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	7.85 min:	401.3 ( <i>M</i> + <i>H</i> <sup>+</sup> ) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Hexane/ <i>tert</i> -butyl methyl ether (4/1)
		Single spot observed, <i>R</i> <sub>f</sub> = 0.3.
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm <sup>-1</sup> , KBr powder
	Peaks:	2927, 2853, 1732, 1672, 1611, 1380, 1334, 1296, 1234, 1170, 1041, 885, 685 cm <sup>-1</sup>
<sup>1</sup> H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	CD <sub>3</sub> CN (1.94 ppm)
	Spectral data:	δ 0.84 (3H, s), 0.88 (3H, t, <i>J</i> = 6.8 Hz), 0.92-1.03 (2H, m), 1.07-1.20 (2H, m), 1.19 (3H, s), 1.25-1.33 (6H, m), 1.34-1.54 (3H, m), 1.55-1.60 (3H, m), 1.61-1.70 (3H, m), 1.73 (1H, dt, <i>J</i> = 12.6, 3.3 Hz), 1.85 (1H, m), 2.02 (1H, ddd, <i>J</i> = 3.2, 4.9, 13.3 Hz), 2.10 (1H, m), 2.21 (1H, m), 2.23-2.28 (3H, m), 2.37-2.46 (2H, m), 4.58 (1H, dd, <i>J</i> = 8.1, 8.9 Hz), 5.63 (1H, s) ppm
<sup>13</sup> C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	CD <sub>3</sub> CN (1.32 ppm)
	Spectral data:	δ 12.4, 14.3, 17.7, 21.4, 23.2, 24.1, 25.8, 28.2, 29.5, 32.2, 32.5, 33.3, 34.6, 35.0, 36.1, 36.6, 37.6, 39.5, 43.4, 51.0, 54.8, 83.0, 124.1, 172.3, 174.4, 199.6 ppm
Melting point:		36 °C
Microanalysis:	Found:	C = 77.7%; H = 10.1% (June, 2020)
	Calculated:	C = 78.0%; H = 10.1% (Calculated for C <sub>26</sub> H <sub>40</sub> O <sub>3</sub> )