



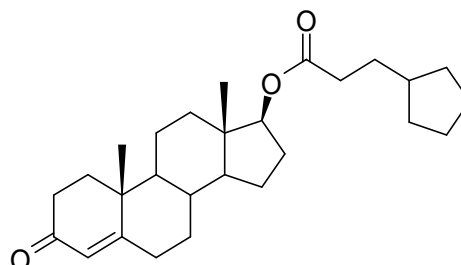
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA S018 Testosterone cypionate

Report ID: S018.2018.03 (Bottle 151221)

Chemical Formula: C<sub>27</sub>H<sub>40</sub>O<sub>3</sub>

Molecular Weight: 412.6 g/mol



## Certified value

Batch No.

CAS No.

Purity (mass fraction)

12-S-06

58-20-8

98.6 ± 1.4%

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

**IUPAC name:** (17β)-3-Oxoandrost-4-en-17-yl 3-cyclopentylpropanoate.

**Expiration of certification:** The property values are valid till 23 July 2023, 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:** Off-white powder prepared by synthesis, 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 three 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.  
18 June 2020

This report supersedes any issued prior to 18 June 2020

**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.

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## 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 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.

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

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 qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18 5 $\mu\text{m}$ (4.6 mm x 150 mm)
	Column oven:	35 $^{\circ}\text{C}$
	Mobile Phase:	Acetonitrile/MilliQ water (85:15)
	Flow rate:	1.2 mL/min
	Detector:	Waters PDA 2998 operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.1% (10 sub samples in duplicate, September 2012)
	Re-analysis:	Mean = 98.8%, s = 0.06% (5 sub samples in duplicate, August 2013)
	Re-analysis:	Mean = 98.9%, s = 0.09% (5 sub samples in duplicate, July 2014)
	Re-analysis:	Mean = 98.9%, s = 0.03% (5 sub samples in duplicate, July 2015)
	Re-analysis:	Mean = 99.0%, s = 0.03% (5 sub samples in duplicate, July 2018)
Thermogravimetric analysis:	Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (September 2012)	
Karl Fischer analysis:	Moisture content < 0.1% mass fraction (September 2012 and August 2013)	
	Moisture content < 0.1% mass fraction (July 2014)	
	Moisture content < 0.1% mass fraction (June 2015)	
	Moisture content < 0.1% mass fraction (June 2018)	

## Spectroscopic and other characterisation data

GC-MS:	Column: TG-1MS, 30 m x 0.25 mm I.D. x 0.25 µm Program: 180 °C (1 min), 30 °C/min to 300 °C (20 min) Injector: 250 °C Transfer line temp: 280 °C Carrier: Helium, 1.0 mL/min Split ratio: 20/1
	The retention time of the parent compound is reported along 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 (13.6 min): 412 (M <sup>+</sup> , 20), 370 (19), 341 (14), 288 (27), 271 (36), 270 (23), 230 (35), 228 (34), 147 (95), 124 (100), 107 (51), 91 (35), 79 (46), 67 (33), 55 (74), 41 (33) <i>m/z</i>
HS-GC-MS:	Instrument: Agilent 6890/5973/G1888 Column: DB-624, 30 m x 0.25 mm I.D. x 1.4 µm 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: Ethyl acetate, hexane, methylcyclopentane
TLC:	Conditions: Kieselgel 60F <sub>254</sub> . Hexane/ethyl acetate (4/1) Single spot observed, R <sub>f</sub> = 0.50. Visualisation with UV at 254 nm
IR:	Instrument: Biorad FTS3000MX FT-IR Range: 4000-400 cm <sup>-1</sup> , KBr powder Peaks: 2966, 2941, 2871, 1728, 1678, 1617, 1451, 1348, 1234, 1120, 1038, 1013, 958, 954, 867, 831, 776, 687, 568, 515, 460 cm <sup>-1</sup>
<sup>1</sup> H NMR:	Instrument: Bruker Avance-400 Field strength: 400 MHz Solvent: CDCl <sub>3</sub> (7.26 ppm) Spectral data: δ 0.84 (3H, s), 0.94 (1H, dt, <i>J</i> = 4.0, 11.9 Hz), 1.01-1.22 (5H, m), 1.19 (3H, s), 1.29-1.45 (2H, m), 1.46-1.80 (15H, m) 1.85 (1H, m), 2.02 (1H, ddd, <i>J</i> = 3.4, 4.8, 13.4 Hz), 2.17 (1H, m), 2.25-2.46 (6H, m), 4.61 (1H, dd, <i>J</i> = 8.0, 9.0 Hz), 5.72 (1H, s) ppm  Ethyl acetate and hexane estimated at 0.02% and 0.1% mass fraction respectively was observed in the <sup>1</sup> H NMR.
<sup>13</sup> C NMR:	Instrument: Bruker Avance-400 Field strength: 101 MHz Solvent: CDCl <sub>3</sub> (77.0 ppm) Spectral data: δ 12.1, 17.4, 20.6, 23.5, 25.1, 27.5, 31.3, 31.5, 32.4, 32.7, 33.87, 33.92, 35.5, 35.7, 36.7, 38.6, 39.7, 42.5, 50.3, 53.8, 82.2, 124.0, 170.8, 173.9, 199.3 ppm
Melting point:	98-100 °C
Microanalysis:	Found: C = 78.8%; H = 10.0% (September, 2012) Calculated: C = 78.6%; H = 9.8% (Calculated for C <sub>27</sub> H <sub>40</sub> O <sub>3</sub> )