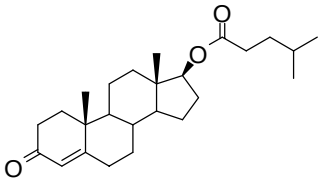
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D688b: Testosterone isocaproate

Report ID: D688b.2020.01 (Bottled 160211)

Chemical Formula: $C_{25}H_{38}O_3$

Molecular Weight: 386.6 g/mol



Property value

Batch No.	CAS No.	Purity estimate
12-S-07	15262-86-9	94.6%

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

Expiration of certification: The property values are valid till 1 April 2023, i.e. three 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 solid 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 reference material is recommended for qualitative analysis only.

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 ºC in a closed container in a dry, dark area.

Stability: This material has demonstrated stability over a minimum period of three years. 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.
14 April 2020

This report supersedes any issued prior to 14 April 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.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. Impurities of related structure were assessed by HPLC with UV detection. The purity estimate was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

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

Supporting evidence is provided by Karl Fischer analysis and ^{1}H NMR spectroscopy qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Waters alliance 2650 or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (85:15 v/v)
	Flow rate:	1.2 mL/min
	Detector:	Waters 2998 PDA operating at Max plot or Shimadzu SPD-M20A PDA operating at Max plot
	Relative peak area of the main component:	
	Initial analysis:	Mean = 93.7%, s = 0.4% (10 sub samples in duplicate, February 2013)
	Re-analysis:	Mean = 94.0%, s = 0.2% (5 sub samples in duplicate, March 2014)
	Re-analysis:	Mean = 94.1%, s = 0.4% (5 sub samples in duplicate, February 2017)
	Re-analysis:	Mean = 94.9%, s = 0.3% (5 sub samples in duplicate, April 2020)
Karl Fischer analysis:		Moisture content ≤ 0.2% mass fraction (March 2013, 2014, 2017 and November 2019)
Thermogravimetric analysis:		Non volatile residue ≤ 0.2% mass fraction (March 2013). The volatile content (e.g.organic solvents and/or water) could not be determined because of the inherent volatility of the material and/or degradation at elevated temperatures.

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro
	Column:	X-Bridge C-18, 100 mm × 4.6 mm I.D. × 5 µm
	Column temp:	40 °C
	Solvent system:	2% 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:	30 µL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	3.5 kV
	Cone voltage:	15 V
	Source temp:	130 ºC
	Desolvation gas temp:	350 ºC
	Cone gas flow rate:	27 L/hr
	Desolvation gas flow:	713 L/hr
	The retention time of testosterone isocaproate is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	6.22 min:	387.4 (M+H+) *m/z*
GC-MS:	Instrument:	Agilent 6890/5973
	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 (9.19 min):	386 (M+, 10), 344 (20), 288 (16), 271 (19), 270 (15), 230 (32), 228 (36), 185 (15), 147 (91), 124 (100), 99 (60), 81 (65), 43 (65) *m/z*
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:	Hexane
TLC:	Conditions:	Kieselgel 60F254. Hexane/ethyl acetate (4/1) Single spot observed, Rf = 0.2. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	2916, 2852, 1735, 1670, 1617, 1273, 1183, 1043, 940, 864 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	CDCl3 (7.26 ppm)
	Spectral data:	δ 0.83 (3H, s), 0.88-1.09 (3H, m), 0.89 (6H, d, J = 6.4 Hz), 1.14-1.22 (1H, m), 1.18 (3H, s), 1.29-1.87 (12H, m), 2.02 (1H, ddd, J = 3.3, 5.0, 13.4 Hz), 2.17 (1H, m), 2.25-2.46 (6H, m), 4.60 (1H, dd, J = 7.8, 9.1 Hz), 5.72 (1H, d, J = 1.0 Hz) ppm Methanol estimated at 0.2% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	CDCl3 (77.2 ppm)
	Spectral data:	δ 11.9, 17.3, 20.4, 22.1, 22.2, 23.4, 26.3, 27.4, 27.5, 31.4, 32.5, 32.6, 33.8, 35.3, 35.6, 36.5, 38.5, 42.4, 50.2, 53.6, 82.1, 123.8, 170.8, 173.9, 199.2 ppm
Melting point:		74-78 ºC
Microanalysis:	Found:	C = 77.6%; H = 10.1% (March, 2013)
	Calculated:	C = 77.7%; H = 9.9% (Calculated for $C_{25}H_{38}O_3$)