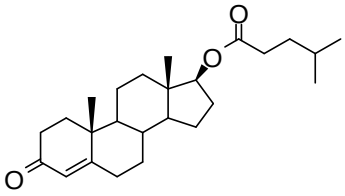
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D688b.2017.01 (Bottled 160211)
This batch of bottles was prepared from the bulk material on 11th February 2016.

Compound Name: **Testosterone isocaproate**
Collection Number: D688b
Chemical Formula: $C_{25}H_{38}O_3$
CAS Number: 15262-86-9
Structure:



Description: Off white solid
Batch Number: 12-S-07
Molecular Weight: 386.6
Release date: 8th May 2013

Purity (mass fraction): 93.4 ± 1.9% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler Or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (85:15)
	Flow rate:	1.2 mL/min
	Detector:	Shimadzu SPD-M20A/Waters 2998 PDA operating at Max plot
	Relative peak area response of 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)
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.
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (March 2013 and 2014) Moisture content 0.2% mass fraction (March 2017)

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 temperature: 350 ºC
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate: 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 60F_{254}. Hexane/ethyl acetate (4/1) Single spot observed, R_f = 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: $CDCl_3$ (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: $CDCl_3$ (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) Calc: C = 77.7%; H = 9.9% (Calculated for $C_{25}H_{38}O_3$)	

Expiration of certification
The property values are valid till 18th February 2020, 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.

The long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of three years from the date of re-certification.

In the absence of 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 stability trials conducted on similar materials by NMI Australia over the last 10 years.

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.

Intended use
For ***in vitro*** laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 14 March, 2017.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 14th March 2017.