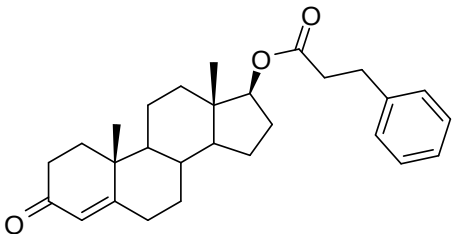
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S030: Testosterone phenylpropionate



Report ID: S030.2022.02 (Bottled 160511)

Chemical Formula: $C_{28}H_{36}O_3$

Molecular Weight: 420.6 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
14-S-07	**1255-49-8**	**98.1 ± 1.4%**

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

IUPAC name: 3-Oxoandrost-4-en-17-yl 3-phenylpropanoate

Expiration of certification: The property values are valid till 21 April 2027, 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 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 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 ºC 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.
17 November 2022

This report supersedes any issued prior to 17 November 2022.

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.

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 singlet at 5.62 ppm, and the one-proton triplet at 4.49 ppm were measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by HPLC with UV/Vis detection at 210 nm, thermogravimetric analysis, Karl-Fischer analysis and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5.0 µm (4.6 mm x 150 mm)
	Column oven:	35 °C
	Mobile Phase:	Acetonitrile/Milli-Q water (85: 15)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 210 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.02% (10 sub samples in duplicate, March 2015)
	Re-analysis:	Mean = 99.4%, s = 0.04% (5 sub samples in duplicate, April 2016)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, May 2017)
	Re-analysis:	Mean = 99.4%, s = 0.06% (5 sub samples in duplicate, May 2018)
	Re-analysis:	Mean = 99.3%, s = 0.02% (5 sub samples in duplicate, June 2019)
	Re-analysis:	Mean = 99.4%, s = 0.004% (5 sub samples in duplicate, April 2022)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (March and April 2015) Moisture content 0.15% mass fraction (May 2017) Moisture content 0.39% mass fraction (April 2018) Moisture content < 0.1% mass fraction (May 2019 and April 2022)
Thermogravimetric analysis:		Volatile content 0.1 % and non-volatile residue 0.3 % mass fraction (March 2015)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethyl terephthalate (100.0% mass fraction)
	Initial analysis:	Mean (5.62 ppm) = 98.2%, s = 0.3% (5 sub samples, April 2015)
	Initial analysis:	Mean (4.49 ppm) = 98.1%, s = 0.3% (5 sub samples, April 2015)

Spectroscopic and other characterisation data

LC-MS:	Parent compound:		
	Instrument:	Waters 2695 (HPLC)/Micromass Quatro	
	Column:	Alltima, 100 mm x 4.6 I.D. x 5.0 µm	
	Column temp:	40°C	
	Solvent System:	0.2 percent formic acid buffered to pH 3 [0.2% v/v], acetonitrile [85% v/v], MilliQ water [14.8% v/v]	
	Flow Rate:	1 mL/min	
	Sample Prep.:	1000 µg/g in acetonitrile/MilliQ water (85:15)	
	Injection volume:	10 µL	
	Ionisation mode:	Electrospray positive ion	
	Capillary voltage:	3.5 kV	Cone voltage: 10 V
	Source temp:	130 °C	Desolvation gas temperature: 350 °C
	Cone gas flow rate:	26 L/hr	Desolvation gas flow rate: 751 L/hr
	The retention time of testosterone phenylpropionate is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	6.21 min:	421.3 ($M+H^+$) m/z	
TLC:	Conditions:	Kieselgel 60F254. Hexane/acetone (4/1) Single spot observed, Rf = 0.40. Visualisation with UV at 254 nm	
IR:	Instrument:	Bruker Alpha FT-IR	
	Range:	4000-400cm^{-1}, neat	
	Peaks:	2942, 1733, 1671, 1416, 1290, 1229, 1162, 1006, 863, 752, 701, 518 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	
	Solvent:	CD_3OD (3.31 ppm)	
	Spectral data:	δ 0.80 (3H, s), 0.94-1.17 (4H, m), 1.23 (3H, s), 1.32-1.49 (3H, m), 1.56-1.72 (5H, m), 1.88 (1H, m), 2.05-2.14 (2H, m), 2.26-2.32 (2H, m), 2.44-2.51 (2H, m), 2.64 (2H, t, *J* = 7.5 Hz), 2.92 (2H, t, *J* = 7.5 Hz), 4.57(1H, dd, *J* = 8.0, 9.5 Hz), 5.71 (1H, s), 7.16-7.27 (5H, m) ppm.	
		Hexane estimated at 0.2% mass fraction was observed in the ^{1}H NMR Ethyl acetate estimated at 0.04% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	125 MHz	
	Solvent:	CD_3OD (49.0 ppm)	
	Spectral data:	δ 12.4, 17.7, 21.6, 24.4, 28.4, 32.1, 32.8, 33.8, 34.7, 36.6, 36.8, 36.9, 37.8, 40.0, 43.7, 51.5, 55.2, 84.0, 124.2, 127.3, 129.4, 129.5, 141.8, 174.7, 174.9, 202.3 ppm.	
Melting point:		115-116 °C	
Microanalysis:	Found:	C = 79.9%; H = 8.8% (March 2015)	
	Calculated:	C = 80.0%; H = 8.6% (Calculated for $C_{28}H_{36}O_3$)	