



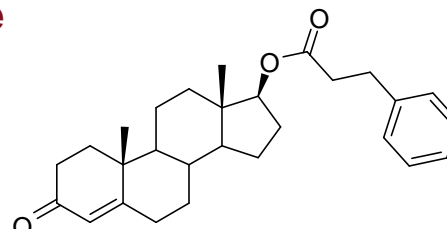
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S030: Testosterone phenylpropionate

Report ID: S030.2019.01 (Bottled 160511)

Chemical Formula: C₂₈H₃₆O₃

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 11 June 2022, 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 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%. 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 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 2019

This report supersedes any issued prior to 18 June 2019

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. 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 μ m (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)

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)

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-d₆ (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 μ m
	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 μ g/g in acetonitrile/MilliQ water (85:15)
	Injection volume:	10 μ L
	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, R _f = 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 ₃ OD (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 ₃ OD (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)