Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

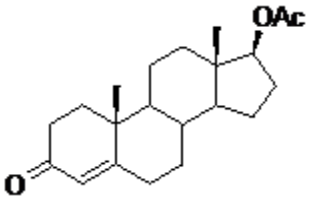
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S042: Testosterone acetate

Report ID: S042.2022.02 (Bottled 181029)

Chemical Formula: $C_{21}H_{30}O_3$

Molecular Weight: 330.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
16-S-05	**1045-69-8**	**99.6 ± 0.5%**

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 acetate

Expiration of certification: The property values are valid till 11 February 2027, i.e. five years from the date of 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: White crystalline 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 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 three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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 GC-FID 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.
1 November 2022

This report supersedes any issued prior to 1 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 mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. 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 HPLC with UV detection, quantitative nuclear magnetic resonance (qNMR), qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 15 °C/min to 260 °C (5 min), 30 °C/min to 300 °C (3 min)
		150 °C (1 min), 10 °C/min to 260 °C (5 min), 30 °C/min to 300 °C (3 min) (2022)
	Injector:	200 °C Detector Temp: 320 °C
	Carrier:	Helium Split ratio: 20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.004% (10 sub samples in duplicate, June 2016)
	Re-analysis:	Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, May 2019)
	Re-analysis:	Mean = 99.8%, s = 0.003% (5 sub samples in duplicate, February 2022)
HPLC:	Instrument:	Thermo Scientific Dionex Ultimate 3000 RS System
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (60:40 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Dionex Ultimate 3000 RS Diode Array Detector at 243 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (6 sub samples in duplicate, July 2016)
Karl Fischer analysis:		Moisture content ≤ 0.2% mass fraction (June 2016, May 2019 and January 2022)
Thermogravimetric analysis:		The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis. The non volatile residue < 0.2% mass fraction (June 2016)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethyl terephthalate (100% mass fraction)
	Initial analysis:	Mean (4.5 ppm) = 99.8%, s = 0.1% (5 sub samples, July 2016)
	Initial analysis:	Mean (5.6 ppm) = 100.0%, s = 0.1% (5 sub samples, July 2016)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 15 °C/min to 260 °C (5 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C Split ratio: 20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	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 (10.0 min):	330 (M^+, 32), 288 (33), 228 (41), 185 (22), 147 (80), 134 (16), 133 (15), 124 (100), 119 (18), 105 (29), 91 (35), 79 (25), 43 (69) *m/z*
ESI-MS:	Instrument:	Waters Acquity TQ API mass spectrometer
	Operation:	Positive ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	50 V
	Peak:	353.1 ($M+Na^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m × 0.25 mm I.D. × 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:	None
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (2;1) Single spot observed, R_f = 0.3. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	2941, 2919, 1736, 1665, 1615, 1227, 1039, 862 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	Benzene-d_6 (7.16 ppm)
	Spectral data:	δ 0.46 (1H, m), 0.55-0.64 (2H, m), 0.69 (3H, s), 0.73 (3H, m), 0.97-1.17 (5H, m), 1.22 (1H, ddd, *J* = 4.6, 14.0, 14.0 Hz), 1.28-1.38 (2H, m), 1.42-1.53 (2H, m), 1.73 (1H, m), 1.74 (3H, s), 1.81-1.92 (2H, m), 2.12-2.20 (2H, m), 2.27 (1H, ddd, *J* = 4.0, 4.0, 16.5 Hz), 4.70 (1H, dd, *J* = 8.2, 8.9 Hz), 5.82 (1H, s) ppm
	Methanol estimated at 0.1% mass fraction was observed in the ^{1}H NMR.	
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	Benzene-d_6 (128.06 ppm)
	Spectral data:	δ 12.2, 17.0, 20.6, 20.7, 23.5, 27.9, 31.5, 32.5, 34.3, 35.3, 35.8, 36.9, 38.3, 42.6, 50.1, 53.6, 82.5, 124.6, 168.4, 170.1, 197.2 ppm
Melting point:		142-143 °C
Microanalysis:	Found:	C = 76.2%; H = 9.2% (June 2016)
	Calculated:	C = 76.3%; H = 9.2% (Calculated for $C_{21}H_{30}O_3$)