Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
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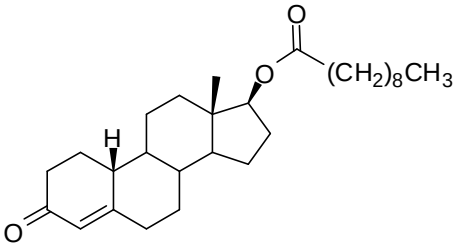
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D684b: Nandrolone decanoate

Report ID: D684b.2020.01

Chemical Formula: $C_{28}H_{44}O_3$

Molecular Weight: 428.7 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
19-S-07	360-70-3	97.5 ± 1.2%

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

IUPAC name: (17β)-3-Oxoestr-4-en-17-yl decanoate.

Expiration of certification: The property values are valid till 29 July 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: Off-white powder sourced from an external supplier, purified 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%.

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 compound in solution has shown signs of degradation to form a hydroxylated species.

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.
28 August 2020

This report supersedes any issued prior to 28 August 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. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, 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 qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters alliance 2695
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (90:10 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A or Waters 2998 PDA operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.7%, s = 0.06% (10 sub samples in duplicate, August 2019)
	Re-analysis:	Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, July 2020)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (August 2019 and July 2020)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (August 2019)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP6890/5973	
	Column:	DB-5MS, 30 m x 0.25 mm I.D. x 0.25 μm	
	Program:	180 °C (1 min), 10 °C/min to 300 °C (17 min)	
	Injector:	250 °C	Split ratio: 20/1
	Transfer line temp:	280 °C	Carrier: Helium
	Scan range:	50-550 *m/z*	
	The retention time of the parent compound is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.		
	Parent (23.0 min):	428 (M^+, 49), 274 (100), 256 (75), 155 (38), 110 (68), 55 (42) *m/z*	
LC-MS:	Instrument:	Waters Alliance/ Micromass Quattro TQ Detector	
	Column:	Alltima C-18, 150 mm × 4.6 mm I.D. × 5 μm	
	Column temp:	33 °C	
	Solvent system:	Acetonitrile/0.2 percent formic acid in MilliQ water (90 : 10 v/v)	
	Flow rate:	1 mL/min	
	Sample prep:	2000 μg/g in mobile phase	
	Injection volume:	5 μL	
	Ionisation mode:	Electrospray positive	
	Capillary voltage:	2.5kV	Cone voltage: 25 V
	Source temp:	120 ºC	Desolvation gas temp: 400 ºC
	Cone gas flow rate:	23 L/hr	Desolvation gas flow: 600 L/hr
	The retention time of nandrolone decanoate is reported along with the major peaks in the mass spectrum. The latter is reported as a mass/charge ratio.		
	24.7 min:	429.2 $(M+H)^+$ *m/z*, 470.3 $(M+H+MeCN)^+$ *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:	Ethyl acetate, hexane, methyl decanoate, diethyl ether	
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (4:1) Single spot observed, R_f = 0.3	
IR:	Instrument:	Bruker Alpha Platinum ATR	
	Range:	4000-400 cm^{-1}, neat	
	Peaks:	2953, 2912, 2849, 1731, 1674, 1325, 1255, 1212, 1173, 1051, 885, 720 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	
	Solvent:	Benzene-d_6 (7.16 ppm)	
	Spectral data:	δ 0.34 (1H, ddd, *J* = 4.0, 10.5, 10.5 Hz), 0.57-0.69 (2H, m), 0.77-0.93 (2H, m), 0.82 (3H, s), 0.91 (3H, t, *J* = 7.0 Hz), 1.02-1.10 (3H, m), 1.24-1.41 (16H, m), 1.54(1H, m), 1.56-1.69 (3H, m), 1.74-1.78 (2H, m), 1.95-2.05 (2H, m), 2.20 (1H, m), 2.24 (2H, t, *J* = 8.0 Hz), 2.32 (1H, dt, *J* = 16.0, 4.5 Hz), 4.77 (1H, dd, *J* = 8.0, 9.0 Hz), 5.92 (1H, s) ppm	
	Ethyl acetate, diethyl ether, methyl decanoate and hexane estimated at 0.05, 0.05, 0.02 and 0.5% mass fraction, respectively, were observed in the ^{1}H NMR. An impurity of related structure estimated at 0.6% 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 (128.0 ppm)	
	Spectral data:	δ 12.3, 14.4, 23.1, 23.5, 25.6, 26.02, 26.8, 27.9, 29.5, 29.7, 29.9, 30.7, 32.3, 34.7, 35.3, 36.8, 37.0, 40.2, 42.2, 42.9, 49.2, 49.4, 82.3, 125.2, 164.0, 173.0, 197.5 ppm	
Melting point:		36-38 °C	
Microanalysis:	Found:	C = 78.5%; H = 10.7% (September 2019)	
	Calculated:	C = 78.5%; H = 10.4% (Calculated for $C_{28}H_{44}O_3$)	