Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

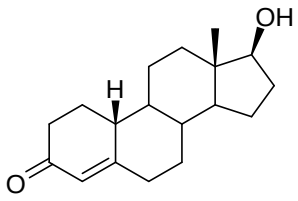
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S038: Nandrolone

Report ID: S038.2019.01

Chemical Formula: $C_{18}H_{26}O_2$

Molecular Weight: 274.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
15-S-013	**434-22-0**	**99.0 ± 0.4%**

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

Synonyms: 17β-Hyroxy-estr-4-en-3-one, 19-Nortestosterone

Expiration of certification: The property values are valid till 3 January 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. 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: White crystalline solid sourced from an external supplier, 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: In the absence of long term 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.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 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.
6 February 2019

This report supersedes any issued prior to 6 February 2019

Characterisation Report:

The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include 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

The certified purity value by qNMR was obtained using the one-proton singlet at 5.8 ppm measured against a certified internal standard of 2,6-di-*t*-butyl-4-methylphenol.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20A 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 (40:60 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA or Waters 2998 PDA operating at 239 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.05% (10 sub samples in duplicate, December 2015)
	Re-analysis:	Mean = 99.3%, s = 0.07% (5 sub samples in duplicate, December 2016)
	Re-analysis:	Mean = 99.2%, s = 0.06% (5 sub samples in duplicate, December 2017)
	Re-analysis:	Mean = 99.3%, s = 0.02% (5 sub samples in duplicate, January 2019)
Thermogravimetric analysis:		Non-volatile residue < 0.2% mass fraction (December 2015). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (November 2015, 2016, 2017 & 2019)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz Solvent: MeOH-d_4 (3.31 ppm)
	Internal standard:	2,6-di-*t*-butyl-4-methylphenol (99.8% mass fraction)
	Initial analysis:	Mean (5.8 ppm) = 99%, s = 0.2% (5 sub samples, January 2016)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 270 °C (5 min), 30 °C/min to 300 °C (2 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 and *bis*-TMS derivative are reported along 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 compound	(10.5 min): 274 (M^+, 100), 256 (22), 231 (22), 215 (28), 147 (26), 110 (74), 91 (45), 79 (35) *m/z*
	Bis-TMS compound	(11.4 min): 418 (M^+, 100), 403 (33), 194 (53), 182 (33), 73 (90) *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 and dichloromethane
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3417, 3373, 2911, 2856, 1662, 1646, 1618, 1333, 1259, 1205, 1052, 1022, 885, 486 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.8 (3H, s), 0.84 (1H, ddd, J = 4.1, 10.7, 22.4 Hz), 0.97-1.15 (3H, m), 1.22-1.40 (3H, m), 1.41-1.65 (3H,m), 1.78-1.91 (3H, m), 2.03-2.13 (2H, m), 2.21-2.31 (3H, m), 2.40 (1H, m), 2.47 (1H, ddd, *J* = 2.5, 4.0, 14.7 Hz), 3.66 (1H, t, *J* = 8.6 Hz), 5.82 (1H, t, *J* = 2.0 Hz) ppm
		Ethyl acetate estimated at 0.4% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 11.2, 23.3, 26.2, 26.7, 30.5, 30.8, 35.6, 36.5, 36.6, 40.6, 42.7, 43.1, 49.7, 49.8, 81.8, 124.7, 166.9, 200.2 ppm
Melting point:		117-118 °C and 125-126 °C (di-morphic crystals, two melting points)
Microanalysis:	Found:	C = 78.7%; H = 9.6% (November, 2015)
	Calculated:	C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)