



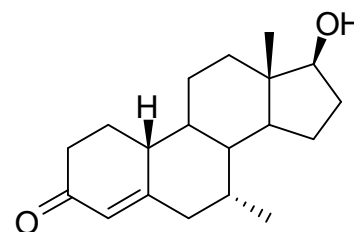
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA S048: 7 $\alpha$ -Methylnandrolone

Report ID: S048.2022.01 (Ampouled 190809)

Chemical Formula: C<sub>19</sub>H<sub>28</sub>O<sub>2</sub>

Molecular Weight: 288.4 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
19-S-03	3764-87-2	980 ± 14 µg

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

**IUPAC name:** (7 $\alpha$ ,17 $\beta$ )-17-Hydroxy-7-methylestr-4-en-3-one.

**Expiration of certification:** The property values are valid till 6 June 2025, 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

**Description:** The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing S048. This material was prepared by synthesis, and certified for identity and purity by NMIA.

**Intended use:** This certified reference material is suitable for use as a primary calibrator.

**Instructions for use:** Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 980 ± 14 µg of anhydrous 7 $\alpha$ -methylnandrolone.

**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%.

**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 ten years. 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 six randomly selected ampoules 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 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.  
20 March 2023

This report supersedes any issued prior to 20 March 2023

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.

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### Characterisation Report:

HPLC:	Instrument:	Waters Alliance 2695 Separations module
	Column:	Alltima C-18, 5 $\mu$ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Methanol/MilliQ water (59:41 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 245 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.01% (6 ampoules in duplicate, September 2019)
	Re- analysis:	Mean = 99.5%, s = 0.03% (5 ampoules in duplicate, August 2020)
	Re- analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, August 2021)
	Re- analysis:	Mean = 99.5%, s = 0.033% (5 ampoules in duplicate, June 2022)

### The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

$I_{\text{ORG}}$  = Organic impurities of related structure,  $I_{\text{VOL}}$  = volatile impurities,  $I_{\text{NVR}}$  = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters Alliance 2695 Separations module
	Column:	Alltima C-18, 5 $\mu$ m (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}$ C
	Mobile Phase:	Methanol / Milli-Q water (55:45 v/v)
	Flow rate:	1.1 mL/min
	Detector:	Waters 2998 PDA operating at 245 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.004% (8 sub samples in duplicate, July 2019)
Karl Fischer analysis:	Moisture content 2.0% mass fraction (April 2019)	
Thermogravimetric analysis:	Volatiles content 2.2% and non-volatile residue < 0.2% mass fraction (April 2019)	

## Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent HP6890/5973
	Column:	DB-5, 30 m $\times$ 0.25 mm I.D. $\times$ 0.25 $\mu$ m
	Program:	200 $^{\circ}$ C (1 min), 15 $^{\circ}$ C/min to 260 $^{\circ}$ C (5 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C,
	Split ratio:	20/1
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 <i>m/z</i>
	The retention times of the parent compound and <i>mono</i> -TMS derivative are 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 (10.0 min):	288 ( $M^+$ , 100), 270 (14), 245 (14), 229 (19), 213 (10), 189 (10), 187 (12), 174 (17), 161 (27), 145 (15), 135 (20), 133 (21), 119 (24), 110 (41), 107 (23), 105 (28), 91 (38), 79 (32), 67 (21), 55 (27), 41 (24) <i>m/z</i>
	<i>Mono</i> -TMS (10.2 min):	360 ( $M^+$ , 13), 345 (11), 270 (100), 255 (11), 229 (20), 213 (12), 174 (13), 161 (12), 129 (84), 115 (15), 105 (13), 91 (16), 73 (62) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Hexane/ethyl acetate (7:3) Single spot observed, $R_f$ = 0.3
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 $\text{cm}^{-1}$ , neat
	Peaks:	3411, 2956, 2941, 2913, 2859, 1662, 1644, 1446, 1385, 1336, 1258, 1212, 1075, 1059, 1022, 902, 882, 764, 611, 546, 493 $\text{cm}^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$\text{CDCl}_3$ (7.26 ppm)
	Spectral data:	$\delta$ 0.75 (3H, d, $J$ = 7.1 Hz), 0.81 (3H, s), 1.04-1.14 (3H, m), 1.21-1.34 (2H, m), 1.42-1.50 (1H, m), 1.50-1.62 (4H, m), 1.82 (1H, dt, $J$ = 12.5, 3.3 Hz), 1.90 (1H, m), 1.97 (1H, m), 2.0-2.04 (1H, m), 2.06-2.12 (1H, m), 2.22-2.31 (3H, m), 2.38-2.43 (1H, m), 2.46 (1H, dd, $J$ = 5.1, 14.0 Hz), 3.67 (1H, t, $J$ = 8.6 Hz), 5.83 (1H, s) ppm Ethyl acetate estimated at 0.12% mass fraction was observed in the <sup>1</sup> H NMR
<sup>13</sup> C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	$\text{CDCl}_3$ (77.16 ppm)
	Spectral data:	$\delta$ 11.2, 13.0, 22.7, 26.8, 26.9, 30.5, 30.8, 36.4, 36.8, 42.5, 42.8, 43.15, 43.22, 43.6, 46.6, 81.9, 126.6, 165.3, 199.8 ppm
Melting point:	139-143 $^{\circ}$ C	
Microanalysis:	Found:	C = 77.6%; H = 9.9% (April 2019)
	Calculated:	C = 79.1%; H = 9.8% (Calculated for $\text{C}_{19}\text{H}_{28}\text{O}_2$ )
		C = 77.6%; H = 9.8% (Calculated for $\text{C}_{19}\text{H}_{28}\text{O}_2 \cdot 0.32\text{H}_2\text{O}$ )