Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

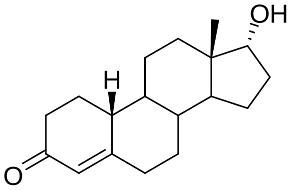
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D722e: 17α-Nandrolone

Report ID: D722e.2023.01 (Ampouled 180621)

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

Molecular Weight: 274.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
18-S-03	**4409-34-1**	**996 ± 14 µg**

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

IUPAC name: (17α)-17-Hydroxyestr-4-en-3-one

Expiration of certification: The property values are valid till 1 September 2028, 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 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D722e. 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. acetontrile). This will transfer 996 ± 14 µg of anhydrous 17α-nandrolone. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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 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 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 seven 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.
6 September 2023

This report supersedes any issued prior to 6 September 2023.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

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: A = MilliQ water; B = Acetonitrile
0-12 min 45% B; 12-15 min 45-80% B; 15-19 min 80% B; 19-21 min 80-45% B; 21-30 min 45% B
Flow rate: 1.0 mL/min
Detector: Shimadzu SPD-M20A or Waters 2998 PDA operating at 243 nm

Relative mass fraction of the main component:
Initial analysis: Mean = 99.5%, s = 0.01% (7 ampoules in duplicate, June 2018)
Re analysis: Mean = 99.4%, s = 0.01% (5 ampoules in duplicate, July 2019)
Re analysis: Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, July 2020)
Re analysis: Mean = 99.4%, s = 0.05% (5 ampoules in duplicate, July 2021)
Re analysis: Mean = 99.4%, s = 0.02% (5 ampoules in duplicate, September 2023)

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.

$\text{Purity} = (100\,\% - I_{ORG}) \times (100\,\% - I_{VOL} - I_{NVR})$ 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
Column: Alltima C-18, 5 µm (4.6 mm x 150 mm)
Column oven: 40 °C
Mobile Phase: A = MilliQ water; B = Acetonitrile
0-12 min 45% B; 12-15 min 45-80% B; 15-19 min 80% B; 19-21 min 80-45% B; 21-30 min 45% B.
Flow rate: 1.0 mL/min
Detector: Shimadzu SPD-M20A PDA operating at 243 nm

Relative mass fraction of the main component:
Initial analysis: Mean = 99.6%, s = 0.03% (10 sub samples in duplicate, June 2018)

Karl Fischer analysis: Moisture content ≤ 0.1% mass fraction (April 2018)

Thermogravimetric analysis: Volatiles content 0.2% and non-volatile residue < 0.2% mass fraction (April 2018)

Spectroscopic and other characterisation data

GC-MS:
Instrument: HP6890/5973
Column: TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program: 180 °C (1 min), 15 °C/min to 300 °C (3 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 isreported 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 (10.4 min): 274 (M^+, 53), 256 (26), 231 (21), 207 (24), 160 (21), 147 (21), 110 (100), 105 (33), 93 (21), 91 (55), 79 (46), 77 (21) *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

TLC:
Conditions: Kieselgel 60F_{254}. Hexane/ethyl acetate (3:2)
Single spot observed, R_f = 0.4.

IR:
Instrument: Biorad FTS3000 MX FT-IR
Range: 4000-400 cm^{-1}, KBr powder
Peaks: 3426, 2965, 2937, 2897, 2860, 1663, 1610, 1447, 1336, 1263, 1202, 1102, 1050, 969, 874 cm^{-1}

^{1}H NMR:
Instrument: Bruker Avance III-500
Field strength: 500 MHz
Solvent: Benzene-d_6 (7.16 ppm)
Spectral data: δ 0.48 (1H, m), 0.51 (3H, s), 0.74-1.02 (5H, m), 1.11 (1H, m), 1.26 (1H, m), 1.32-1.54 (6H, m), 1.60 (1H, m), 1.69-1.82 (2H, m), 1.94-2.05 (3H, m), 2.32 (1H, dt, J = 16.0, 4.5 Hz), 3.51 (1H, dd, J = 3.5, 5.5 Hz), 5.91 (1H, s) ppm

Ethyl acetate 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 (128.1 ppm)
Spectral data: δ 17.2, 24.7, 26.2, 26.8, 31.4, 31.8, 32.9, 35.5, 36.9, 40.9, 42.4, 45.5, 47.5, 49.3, 79.5, 125.1, 164.7, 197.9 ppm

Melting point: 150-151 °C

Microanalysis:
Found: C = 78.9%; H = 9.9% (November 2015)
Calculated: C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)