Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

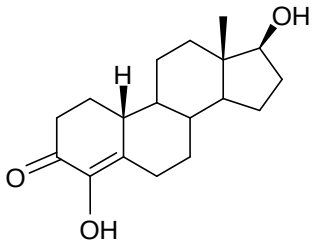
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D853c: 4-Hydroxynandrolone

Report ID: D853c.2023.01 (Ampouled 150603)

Chemical Formula: $C_{18}H_{26}O_3$

Molecular Weight: 290.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
15-S-01	**4721-69-1**	**990 ± 24 µg**

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

IUPAC name: (17β)-4,17-Dihydroxyestr-4-en-3-one.

Expiration of certification: The property values are valid till 18 August 2028, five 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. 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: 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 D853c. 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. acetonitrile). This will transfer 990 ± 24 µg of anhydrous 4-hydroxynandrolone.

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 five 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.
22 August 2023

This report supersedes any issued prior to 22 August 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.

Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water A = MilliQ water; B = Acetonitrile 0-32 min 30%B, 32-34 min 30%-80%B, 34-38 min 80%B, 38-40 min 80%-30%B, 40-45 min 30%B or 0-20 min 30%B, 20-22 min 30%-80%B, 22-27 min 80%B, 27-29 min 80%-30%B, 29-35 min 30%B
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA or Waters PDA 2998 operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (7 ampoules in duplicate, June 2015)
	Re-analysis:	Mean = 99.9%, s = 0.04% (5 ampoules in duplicate, May 2016)
	Re-analysis:	Mean = 99.8%, s = 0.04% (5 ampoules in duplicate, May 2019)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, August 2023)

Note: Estradiol was identified as the major impurity in this material. Due to a significant difference in the extinction coefficient of estradiol and 4-hydroxynandrolone, the impurity was quantified using ^{1}H NMR spectroscopy. The reported mass fraction of the main component in the HPLC-UV analysis does not include the estradiol.

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

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

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

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 30% A for 32 minutes, increase A to 80% in 2 minutes, 80% A for 4 minutes, decrease A to 30% in 2 minutes, 30% A for 5 minutes.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.04% (10 sub samples in duplicate, February 2015)
Karl Fischer analysis:		Moisture content 0.2 % mass fraction (March 2015)
Thermogravimetric analysis:		Volatile content 0.5% and non volatile residue < 0.2% mass fraction (March 2015)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	TG1-MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 250 °C, 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 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 (11.8min):	290 (M^+, 100), 272 (26), 243 (8), 218 (9), 177 (12), 161 (14), 147 (38), 126 (56), 91 (37), 79 (35), 67 (26), 55 (26) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1) Single spot observed, R_f = 0.4. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3542, 3417, 2947, 2913, 1664, 1650, 1653, 1622, 1389, 1351, 1158, 1063, 1052, 876, 618 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.80 (3H, s), 0.82 (1H, ddd, *J* = 3.8, 10.5, 10.5 Hz), 0.94-1.03 (2H, m), 1.12 (1H, m), 1.20-1.36 (3H, m), 1.42-1.52 (3H, m), 1.64 (1H, m), 1.80-1.94 (4H, m), 2.04-2.17 (2H, m), 2.25 (1H, m), 2.35 (1H, m), 2.56 (1H, ddd, *J* = 4.1, 4.1, 17.1 Hz), 3.12 (1H, ddd, *J* = 1.9, 3.7, 15.2 Hz), 3.67 (1H, t, *J* = 8.7 Hz), 6.09 (1H s) ppm
	Ethyl acetate and estradiol were quantified at 0.6% and 0.3% mass fraction respectively 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, 23.4, 26.06, 26.10, 26.3, 29.6, 30.6, 34.7, 36.6, 40.1, 41.7, 43.1, 49.7, 49.9, 81.9, 135.5, 142.2, 194.4 ppm
Melting point:		188-190 °C
Microanalysis:	Found:	C = 74.3%; H = 9.2% (March 2015)
	Calculated:	C = 74.5%; H = 9.0% (Calculated for $C_{18}H_{26}O_3$)