Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

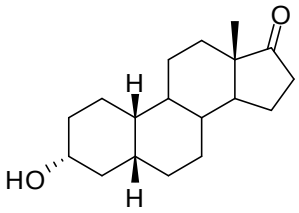
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D554: 19-Noretiocholanolone

Report ID: D554.2022.01 (Ampouled 210218)

Chemical Formula: $C_{18}H_{28}O_2$

Molecular Weight: 276.4 g/mol (HCl)



Certified value

Batch No.	CAS No.	Mass per ampoule
98-002317	**33036-33-8**	**1006 ± 14 µg**

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

IUPAC name: (3α,5β)-3-Hydroxyestran-17-one. (Metabolite of nandrolone (19-nortestosterone))

Expiration of certification: The property values are valid till 20 September 2027, i.e. 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D554. This material was sourced from an external supplier, 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 1006 ± 14 µg of anhydrous 19-noretiocholanolone. 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 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 GC-FID 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.
23 September 2022

This report supersedes any issued prior to 23 September 2022

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:

GC-FID:	Instrument:	Agilent 7890 /8890
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 4 °C/min to 220 °C (6 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.004% (7 ampoules in duplicate, February 2021)
	Re-analysis:	Mean = 99.8%, s = 0.003% (5 ampoules in duplicate, October 2021)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 ampoules in duplicate, September 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 GC-FID, 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 qualitative elemental microanalysis.

GC-FID:	Instrument:	Agilent 7890 and Varian CP-3800
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 4 °C/min to 220 °C (6 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, February 2014)
	Re-analysis:	Mean = 99.8%, s = 0.003% (7 sub samples in duplicate, February 2021)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 250 °C (5 min), 30 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02 (10 samples in duplicate, November 2001)
	Re-analysis:	Mean = 99.7%, s = 0.01 (7 sub samples in duplicate, April 2008)
Karl Fischer analysis:		Moisture content 0.13% mass fraction (April 2008) Moisture content < 0.1% mass fraction (February 2014) Moisture content ≤ 0.1% mass fraction (March 2021)
Thermogravimetric analysis:		Volatiles content 0.2% and non-volatile residue < 0.2% mass fraction (April 2008)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 2, 17 m × 0.20 mm I.D. × 0.10 μm
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm
	Program:	170 °C (0.5 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (2 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention time of the parent compound and *bis*-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 (5.1 min):	276 (M^+, 100), 232 (65), 214 (44), 202 (53), 201 (49), 91 (49) *m/z*
	Bis-TMS (9.1 min):	420 (M^+, 34), 405 (47), 315 (19), 225 (7), 169 (20), 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (6:4) Single spot observed, R_f = 0.3 (5 samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3307, 1738, 1454, 1373, 1041 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance 300
	Field strength:	300 MHz
	Solvent:	$CDCl_3$ (7.27 ppm)
	Spectral data:	δ 0.87 (3H, s), 3.63 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker Avance 300
	Field strength:	75 MHz
	Solvent:	$CDCl_3$ (77.49 ppm)
	Spectral data:	δ 14.2, 22.0, 25.3, 25.5, 26.3, 29.9, 31.7, 32.0, 36.0, 36.3, 36.6, 39.0, 40.3, 41.6, 48.3, 50.9, 71.8, 221.0 ppm
Melting point:		164-166 °C
Microanalysis:	Found:	C = 78.0%; H = 10.4% (July 1998)
	Calculated:	C = 78.2%; H = 10.2% (Calculated for $C_{18}H_{28}O_2$)