Australian Government
Department of Industry, Science and Resources

National Measurement Institute

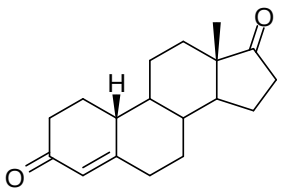
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D721: 19-Norandrostendione

Report ID: D721.2021.03

Chemical Formula: $C_{18}H_{24}O_2$

Molecular Weight: 272.2 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
02-S-03	**734-32-7**	**99.7 ± 0.3%**

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

IUPAC name: Estr-4-ene-3,17-dione

Expiration of certification: The property values are valid till 2 February 2031, i.e. ten 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.

Description: White powder prepared by synthesis, 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: This material has demonstrated stability over a minimum period of ten 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 five 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.
10 November 2022

This report supersedes any issued prior to 10 November 2022.

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

Characterisation Report:

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.

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.

Supporting evidence is provided by qualitative elemental microanalysis.

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.**

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1 (30 m × 0.32 mm × 0.25 μm)
	Program:	220 °C (1 min), 4 °C/min to 280 °C, 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.03% (10 sub samples in duplicate, February 2002)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, March 2008)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 sub samples in duplicate, March 2011)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, April 2016)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, February 2021)
HPLC:	Instrument:	Waters
	Column:	Waters Nova-Pak C18, 5 μm (4.6 mm x 150 mm)
	Column oven:	55 °C
	Mobile Phase:	Acetonitrile/MilliQ water (55:45 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Peak area percentage of total: > 99.9% (3 sub samples in duplicate)
Karl Fischer analysis:		Moisture content ≤ 0.2% mass fraction (March 2008, March 2011, February 2016 and January 2021)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (February 2002 & April 2005)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	ZB-5, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	140 °C (1 min), 40 °C /min to 250 °C, 30 °C /min to 300 °C (3 min)
	Injector:	280 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	HP 6890/5973
	Column:	Ultra 1, 17 m x 0.2 mm I.D.x 0.11 μm
	Program:	189 °C (0.2 min), 3 °C /min to 240 °C, 10 °C /min to 265°C, 30 °C/min to 310 °C (2 min)
	Injector:	250 °C
	Split ratio:	14/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times 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 (17.2 min):	272 (M^+, 100), 244 (18), 228 (28), 186 (36), 110 (48), 91 (55), 79 (45) *m/z*
	Bis-TMS (10.5 min):	416 (M^+, 100), 401 (8), 234 (6), 194 (10), 75 (16), 73 (70), 45 (7) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4:1) Single spot observed, R_f = 0.32. Visualisation with UV light (254 nm)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	1742, 1672, 1620, 1449, 1373, 1366, 1335, 1258, 1204, 1043, 885 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.89 (1H, m), 0.91 (3H, s),1.09-1.17 (1H, m),1.2-1.34 (3H, m), 1.81-1.83 (1H,m), 1.89-1.98 (3H, m), 2.04-2.14 (2H, m), 2.21-2.31 (3H, m), 2.36-2.52 (3H, m), 5.82 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75.5 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 13.7, 21.5, 25.6, 26.5, 29.8, 31.2, 35.2, 35.6, 36.4, 39.8, 42.4, 47.6, 49.5, 50.1, 124.8, 165.6, 199.5, 220.2 ppm
Melting point:		170-171 °C
Microanalysis:	Found:	C = 79.8%, H = 9.2% (February 2002)
	Calculated:	C = 79.4%, H = 8.9% (Calculated for $C_{18}H_{24}O_2$)