Australian Government
Department of Industry, Science and Resources

National Measurement Institute

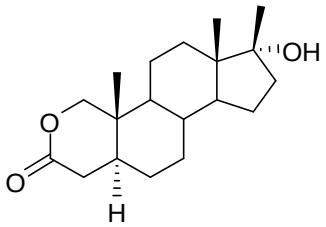
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D620: 17-Epioxandrolone

Report ID: D620.2021.02 (Ampouled 140814)

Chemical Formula: $C_{19}H_{30}O_3$

Molecular Weight: 306.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-S-25	**26624-15-7**	**998 ± 16 µg**

IUPAC name: (4aS,4bS,6aS,7R,9aS,9bR,11aS)-7-Hydroxy-4a,6a,7-trimethyltetradecahydroindeno[4,5-h]isochromen-2(1H)-one

Expiration of certification: The property values are valid till 30 March 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 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 reference material is intended for a single use to prepare a standard solution containing D620. The 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. chloroform). This will transfer approximately 998 µg of anhydrous 17-epioxandrolone. 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.

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 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.
3 November 2022

This report supersedes any issued prior to 3 November 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:

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 I.D. × 0.25 μm		
	Program:	180 °C (1 min), 10 °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 = 99.3%, s = 0.01 % (7 ampoules in duplicate, August 2014)		
	Re-analysis:	Mean = 99.3%, s = 0.03 % (5 ampoules in duplicate, July 2015)		
	Re-analysis:	Mean = 99.1%, s = 0.02 % (5 ampoules in duplicate, June 2018)		
	Re-analysis:	Mean = 99.2%, s = 0.03 % (5 ampoules in duplicate, March 2021)		

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 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 elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890		
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm		
	Program:	180 °C (1 min), 10 °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 = 98.3%, s = 0.06% (10 subsamples, November 1999)		
	Re-analysis:	Mean = 98.8%, s = 0.06% (8 subsamples in duplicate, June 2006)		
	Re-analysis:	Mean = 99.3%, s = 0.004% (7 subsamples in duplicate, August 2014)		
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (September 2013)		
Thermogravimetric analysis:		Volatile content < 0.1% and non-volatile residue < 0.2% mass fraction (January 2000 and June 2006)		

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11 μm
	Program:	140 °C (1 min), 8 °C/min to 250 °C, 30 °C/min to 300 °C (3 min)
	Injector:	280 °C
	Split ratio:	Split less
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Mono-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 (3 min)
	Injector:	280 °C
	Split ratio:	20/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times of the parent compound and *mono*-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 (13.7 min):	306 (M^+, 13), 291 (92), 273 (100), 248 (65), 93 (55) *m/z*
	Mono-TMS (11.0 min):	378 (M^+, 4), 363 (16), 321 (11), 308 (19), 143 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/dichloromethane (50:50) Single spot observed, R_f = 0.44
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3487, 2866, 1712, 1372, 1234, 1208, 1145, 1082 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.66 (3H, s), 0.98 (3H, s), 1.17 (3H, s), 2.20 (1H, dd, *J* = 13.1, 18.8 Hz), 2.49 (1H, dd, *J* = 5.9, 18.8 Hz), 3.90 (1H, dd, *J* = 0.6, 10.7 Hz), 4.22 (1H, d, *J* = 10.7 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77 ppm)
	Spectral data:	δ 10.1, 15.7, 20.8, 22.6, 23.8, 27.1, 29.4, 31.1, 33.7, 34.6, 35.1, 38.1, 40.3, 46.4, 49.3, 49.4, 81.0, 82.0, 170.6 ppm
Melting point:		197-199 °C
Microanalysis:	Found:	C = 74.6%, H = 9.9%
	Calculated:	C = 74.5%, H = 9.9% (Calculated for $C_{19}H_{30}O_3$)