Australian Government
Department of Industry, Science and Resources

National Measurement Institute

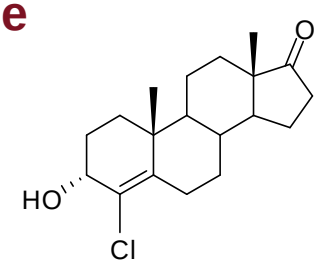
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D563: 4-Chloro-4-androsten-3α-ol-17-one

Report ID: D563.2020.03 (Ampouled 100119)

Chemical Formula: $C_{19}H_{27}ClO_2$

Molecular Weight: 322.9 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
99-000014	51348-73-3	989 ± 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)-4-Chloro-3-hydroxyandrost-4-en-17-one.

Expiration of certification: The property values are valid till 30 September 2025, 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D563. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This certified reference material may be used for instrument calibration

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 989 ± 14 µg of anhydrous 4-chloro-4-androsten-3α-ol-17-one. 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%. 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 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 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.
1 November 2022

This report supersedes any issued prior to 1 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:

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 250 °C (3 min), 20 °C/min to 300 °C (5 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 98.7%, s = 0.01% (7 ampoules in duplicate, January 2010)	
	Re-analysis:	Mean = 98.7%, s = 0.02% (5 ampoules in duplicate, December 2012)	
	Re-analysis:	Mean = 98.7%, s = 0.02% (5 ampoules in duplicate, December 2015)	
	Re-analysis:	Mean = 98.7%, s = 0.02% (5 ampoules in duplicate, Stember 2020)	

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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

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

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

Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm	
	Program:	180 °C (1 min), 10 °C/min to 250 °C (3 min), 20 °C/min to 300 °C (5 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 = 0.04% (7 sub samples in duplicate, February 1999)	
	Re-analysis:	Mean = 98.7%, s = 0.03% (3 sub samples in duplicate, May 2003)	
	Re-analysis:	Mean = 98.6%, s = 0.1% (5 sub samples in duplicate, July 2006)	
	Re-analysis:	Mean = 98.7%, s = 0.01% (7 sub samples in duplicable, January 2010)	
Thermogravimetric analysis:		Volatiles content and non-volatile residue < 0.3% mass fraction (March 1999)	
Karl Fischer analysis:		Moisture content 0.2% mass fraction (February 2010)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP6890/5973	
Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 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 inj. (20/1)
Transfer line temp:	300 °C	Carrier: Helium, 1.0 mL/min
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	
Injector:	280 °C	Transfer line temp: 300 °C
Carrier:	Helium	Split inj. (20/1)
Scan range:	50-550 m/z	

The retention times of the parent material and its *bis*-TMS derivative are reported along 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 (6.5 min): 322 (M^+, 20), 287 (100), 269 (57), 150 (29), 105 (30), 91 (27) *m/z*
Bis -TMS (11.4 min): 466 (M^+, 67), 451 (63), 431 (43), 221 (16), 169 (22), 73 (100) *m/z*
The *bis*-silylated derivative of the synthetic material co-elutes with a derivatised comparison sample of 4-chloro-4-androsten-3α-ol-17-one and the two materials produce matching mass spectra.

HPLC: Peak area percentage of total: > 99% (3 sub samples)

Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
Mobile Phase:	Acetonitrile/water (60:40)
Flow Rate:	1 mL/min
Detector:	Refractive index

TLC: Conditions: Kieselgel 60F_{254}. Hexane/ethyl acetate/chloroform (15:10:5)
Single spot observed, R_f = 0.30 (5 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3442, 1725, 1450, 1387, 1063, 1011, 779 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Advance-300	
Field strength:	300 MHz	Solvent: $CDCl_3$/DMSO-d_6 (7.26 ppm/2.5 ppm)
Key spectral data:	δ 0.89 (3H, s), 1.06 (3H, s), 4.10 (1H, br s) ppm	

^{13}C NMR:

Instrument:	Bruker Advance-300	
Field strength:	75 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 14.0, 18.3, 21.1, 22.0, 26.6, 28.5, 30.5, 31.7, 31.7, 35.3, 36.0, 40.7, 47.8, 51.2, 54.5, 69.4, 128.2, 142.7, (221) ppm	

Melting point: 239-241 °C

Microanalysis:

Found:	C = 70.7%; H = 8.2%; Cl = 11.2% (February 1999)
Calculated:	C = 70.7%; H = 8.4%; Cl = 11.0% (Calculated for $C_{19}H_{27}ClO_2$)