Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

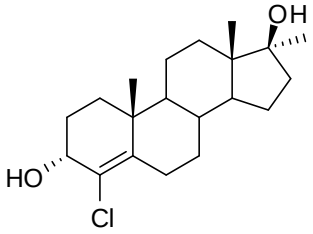
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S044: 17α-Methyl-4-chloroandrost-4-ene-3α,17β-diol

Report ID: S044.2020.01 (Ampouled 171005)

Chemical Formula: $C_{20}H_{31}ClO_2$

Molecular Weight: 338.9 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
17-S-04	**35937-40-7**	**972 ± 20 μg**

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

IUPAC name: (3β,17β)-4-Chloro-17-methylandrost-4-ene-3,17-diol.

Expiration of certification: The property values are valid till 6 August 2023, i.e. three 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: 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 S044. 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. chloroforml). This will transfer 972 ± 20 μg of anhydrous 17α-methyl-4-chloroandrost-4-ene-3α,17β-diol.

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: This material has demonstrated stability over a minimum period of three 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 September 2020

This report supersedes any issued prior to 1 September 2020

Characterisation Report:

GC-FID:	Instrument:	Varian CP-3800 or Agilent 7890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 280 °C (8 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *Bis*-TMS derivative:	
	Initial analysis:	Mean = 99.4%, s = 0.01% (7 ampoules in duplicate, November 2017)
	Re-analysis:	Mean = 99.3%, s = 0.05% (5 ampoules in duplicate, September 2018)
	Re-analysis:	Mean = 99.2%, s = 0.01% (5 ampoules in duplicate, August 2019)
	Re-analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, August 2020)

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 headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1MS, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 280 °C (8 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *Bis*-TMS derivative	
	Initial analysis:	Mean = 99.3%, s = 0.01% (7 sub samples in duplicate, August 2017)
Karl Fischer analysis:		Moisture content 0.1% mass fraction (August 2017)
Thermogravimetric analysis:		Volatiles content 2.6% and non-volatile residue < 0.2% mass fraction (August 2017)

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973
Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 15 °C/min to 240 °C (12 min), 30 °C/min 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*

Bis-TMS derivative:

Instrument:	Shimadzu GCMS-QP2010 Ultra
Column:	HP Ultra 1, 25 m x 0.22 mm I.D. x 0.11 µm
Program:	115 °C (0.8 min), 90 °C/min to 180 °C, 5 °C/min to 190 °C, 3 °C/min to 230 °C, 10 °C/min to 265 °C, 30 °C/min to 320 °C (4 min)
Injector:	250 °C, Split ratio: 20/1
Transfer line temp:	250 °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 (14.5 min):	304 (29), 302 (83), 289 (28), 287 (76), 267 (38), 251 (21), 227 (10), 191 (24), 179 (24), 177 (25), 161 (37), 143 (25), 141 (31), 133 (36), 131 (34), 128 (32), 121 (30), 119 (36), 115 (38), 107 (52), 105 (100), 91 (88), 79 (45), 77 (44) *m/z*
Bis-TMS (18.8 min):	482 (M^+, < 1), 467 (2), 447 (12), 357 (47), 341 (3), 267 (5),143 (100), 130 (12), 75 (20), 73 (46)*m/z*

ESI-MS:

Instrument:	Micromass Quatro LC Micro
Operation:	Negative ion mode, direct infusion at 20 µL/min
Ionisation:	ESI spray voltage at 3.5 kV positive ion
EM voltage:	500 V
Cone voltage:	40 V
Peak:	337.2 ($M\text{-}Cl^{35}\text{-}H^+$), 339.1($M\text{-}Cl^{37}\text{-}H^+$) *m/z*

HS-GC-MS:

Instrument:	Agilent 6890/5973/G1888
Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 µm
Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
Injector:	150 °C
Transfer line temp:	280 °C Split ratio: 50/1
Carrier:	Helium, 1.2 mL/min
Solvents detected:	No solvents detected

TLC:

Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (1:1) Single spot observed, R_f = 0.74. Visualisation with vanillin.

IR:

Instrument:	Bruker Alpha Platinum ATR
Range:	4000-400 cm^{-1}, neat
Peaks:	3254, 2971, 2932, 2874, 2847, 1450, 1373, 1312, 1290, 1150, 1084, 943, 819, 753, 705, 649, 551 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III-500
Field strength:	500 MHz
Solvent:	$CDCl_3$ (7.26ppm)
Spectral data:	δ 0.81-0.94 (2H, m), 0.88 (3H, s), 1.05 (3H, s), 1.15 (1H, m), 1.19 (3H, s), 1.24-1.33 (2H, m), 1.38 (1H, ddd, J = 3.8, 12.9, 26.9 Hz), 1.49-1.65 (6H, m), 1.71-1.88 (5H, m), 1.93 (1H, dddd, *J* = 2.1, 4.7, 14.3, 14.3 Hz), 2.93 (1H, dddd, *J* = 2.8, 4.2, 14.3 Hz), 4.14 (1H, m) ppm

Methanol estimated at 2.4% mass fraction was observed in the ^{1}H NMR.

^{13}C NMR: Instrument: Bruker Avance III-500 Field strength: 126 MHz
Solvent: $CDCl_3$ (77.19 ppm)
Spectral data: δ 14.1, 18.3, 21.4, 23.4, 26.0, 26.8, 27.6, 31.2, 31.7, 31.8, 36.5, 39.1, 40.7, 45.5, 50.4, 54.5, 70.0, 81.8, 127.2, 143.9 ppm

Melting point: 218-219 °C

Microanalysis:

Found:	C = 69.9%; H = 9.4%; Cl% = 10.2% (August 2017)
Calculated:	C = 70.1%; H = 9.3%; Cl% = 10.2% (Calculated for $C_{20}H_{31}ClO_2$ with 2.4% methanol)