Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

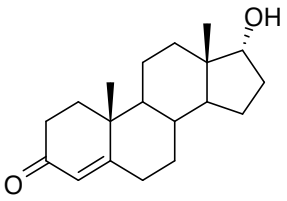
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D547b: Epitestosterone

Report ID: D547b.2022.01 (Ampouled 200908)

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
16-S-07	**481-30-1**	**995 ± 28 µg**

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

IUPAC name: (17α)-17-Hydroxyandrost-4-en-3-one.

Expiration of certification: The property values are valid till 10 August 2025, 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 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 intended for a single use to prepare a standard solution containing D547b. This material was prepared by synthesis, 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 995 ± 28 µg of anhydrous Epitestosterone. 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: 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 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.
12 September 2022

This report supersedes any issued prior to 12 September 2022.

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

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see http://www.bipm.org).

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-1MS, 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 (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 = 99.5%, s = 0.01% (7 ampoules in duplicate, September 2020)
	Re-analysis:	Mean = 99.5%, s = 0.04% (5 ampoules in duplicate, August 2021)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, August 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 from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include 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

The certified purity value by qNMR was obtained using the one-proton singlet at 5.6 ppm measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by elemental microanalysis.

Note: Epitestosterone has been shown to be sensitive to the quality of the GC liner. Injection onto a dirty liner may result in degradation leading to artefact formation.

GC-FID:	Instrument:	Agilent 6890/7890	
	Column:	HP-1, 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 (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 = 99.5%, s = 0.004% (10 sub samples in duplicate, February 2017)	
	Re-analysis:	Mean = 99.5%, s = 0.005% (5 sub samples in duplicate, January 2018)	
	Re-analysis:	Mean = 99.5%, s = 0.008% (5 sub samples in duplicate, September 2020)	
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (February 2017). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis.	
Karl Fischer analysis:		Moisture content 0.3% mass fraction (March 2017) Moisture content < 0.1% mass fraction (January 2018, September 2020)	
QNMR:	Instrument:	Bruker Avance-III-500	
	Field strength:	500 MHz	
	Solvent:	DMSO-d_6 (2.50 ppm)	
	Internal standard:	Dimethyl terephthalate (100% mass fraction)	
	Initial analysis:	Mean (5.62 ppm) = 98.7%, s = 0.15% (5 sub samples, March 2017)	

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Waters Acquity TQ API mass spectrometer
	Operation:	Positive ion mode, direct infusion at 10 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	650 V Cone voltage: 20 V
	Peak:	311.1 ($M+Na^+$) *m/z*
	Operation:	Negative ion mode, direct infusion at 10 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV negative ion
	EM voltage:	650 V Cone voltage: 40 V
	Peak:	287.2 ($M-H^+$) m/z
GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	200 °C (1 min), 15 °C/min to 260 °C (5 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C Transfer line temp: 280 °C
	Carrier:	Helium, 1.0 mL/min Split ratio: 20/1
	The retention times of the parent compound and *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 (8.5 min):	288 (M+, 42), 270 (28), 246 (25), 228 (41), 203 (28), 187 (10), 165 (19), 159 (15), 147 (100), 133 (25), 131 (26), 124 (96), 109 (32), 105 (46), 91 (69), 79 (52), 77 (37), 67 (30), 55 (32), 41 (29) *m/z*
	Bis-TMS (8.9 min):	434 (M^+, 15), 432 (100), 209 (10), 208 (10), 75 (23), 73 (56) *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
	Carrier:	Helium, 1.2 mL/min Split ratio: 50/1
	Solvents detected:	Ethyl acetate
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20) Single spot observed, R_f = 0.2. Visualisation with UV at 254 nm
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm-1, KBr pellet
	Peaks:	3420, 1654, 1610, 1380, 1229, 1190, 872 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26ppm)
	Spectral data:	δ 0.71 (3H, s), 0.97 (1H, m), 1.11 (1H, m), 1.17-1.25 (1H, m), 1.19 (3H, s), 1.38-1.53 (4H, m), 1.53-1.60 (2H, m), 1.65 (1H, m), 1.71 (1H, m), 1.79 (1H, m), 1.88 (1H, m), 2.04 (1H, m), 2.18 (1H, m), 2.27 (1H, m), 2.32-2.46 (3H, m), 3.76 (1H, d, *J* = 5.9 Hz), 5.73 (1H, s) ppm
	Ethyl acetate estimated at 0.2% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.16 ppm)
	Spectral data:	δ 17.0, 17.6, 20.7, 24.7, 31.3, 32.4, 32.5, 33.1, 34.1, 35.9, 36.0, 38.8, 45.3, 48.3, 53.7, 79.8, 124.0, 171.5, 199.8 ppm
Melting point:		222-224 °C
Microanalysis:	Found:	C = 79.3%; H = 10.2% (March 2017)
	Calculated:	C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)