



Australian Government
Department of Industry,
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National
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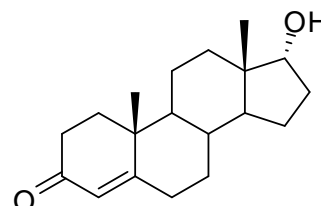
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D547: Epitestosterone

Report ID: D547.2020.01 (Ampouled 120918)

Chemical Formula: C₁₉H₂₈O₂

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-000702	481-30-1	949 ± 34 µg

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

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

Expiration of certification: The property values are valid till 1 May 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 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D547. 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 949 ± 34 µg of anhydrous *epi*-testosterone. 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 °C 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 three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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 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.
18 May 2020.

This report supersedes any issued prior to 18 May 2020.

NATA logo notice: Accredited for compliance with ISO Guide 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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 6890
	Column:	HP-1 Capillary, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 10 °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 = 97.6%, s = 0.03% (7 ampoules in duplicate, September 2012)
	Re-analysis:	Mean = 97.7%, s = 0.01% (5 ampoules in duplicate, July 2013)
	Re-analysis:	Mean = 97.5%, s = 0.07% (5 ampoules in duplicate, June 2014)
	Re-analysis:	Mean = 97.8%, s = 0.01% (5 ampoules in duplicate, June 2015)
	Re-analysis:	Mean = 97.7%, s = 0.08% (5 ampoules in duplicate, May 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 quantitative NMR and mass balance from a combination of traditional analytical techniques, including GC-FID and thermogravimetric analysis. The purity value is calculated as per Equation 1.

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

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

The purity estimate by QNMR was obtained using a combination of peaks against a certified internal standard of dimethyl sulfone.

Supporting evidence is provided by elemental microanalysis and ¹H NMR spectroscopy.

QNMR:	Instrument:	Bruker DMX-400
	Field strength:	400 MHz
	Solvent:	MeOH- <i>d</i> ₄
	Internal standard:	Dimethyl sulfone (100% mass fraction)
	Initial Analysis:	Mean (0.7 ppm) = 94.0%, s = 0.3 % (5 sub samples, November 2008)
	Initial Analysis:	Mean (0.5-0.9 ppm) = 94.9%, s = 0.3 % (5 sub samples, November 2008)
	Initial Analysis:	Mean (5.4-6.4 ppm) = 96.1%, s = 0.2 % (5 sub samples, November 2008)
	Initial Analysis:	Mean (5.72 ppm) = 94.8%, s = 0.3 % (5 sub samples, November 2008)
	Initial Analysis:	Mean (1.92 ppm) = 94.1%, s = 0.5 % (5 sub samples, November 2008)

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1 Capillary, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	200 °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 = 97.6%, s = 0.12% (10 sub samples in duplicate, November 2001)
	Re-analysis:	Mean = 97.7%, s = 0.02% (7 sub samples in duplicate, July 2007)

HPLC:	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/water (65:35)
	Flow Rate:	1.0 mL/min
	Detector:	Refractive index
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98% (3 sub samples)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (April 1999, July 2004, August 2005 and July 2007)

Spectroscopic and other characterisation data

GC-MS:	<p>Parent compound:</p> <p>Instrument: HP6890/5973</p> <p>Columns: HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11 μm</p> <p>Program: 190 $^{\circ}$C (1 min), 12 $^{\circ}$C/min to 300 $^{\circ}$C (3 min)</p> <p>Injector: 280 $^{\circ}$C</p> <p>Transfer line temp: 300 $^{\circ}$C</p> <p>Carrier: Helium, 1.0 mL/min</p> <p>Splitless injection</p> <p><i>Bis</i>-TMS derivative:</p> <p>Instrument: HP6890/5973</p> <p>Columns: HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μm</p> <p>Program: 170 $^{\circ}$C (1 min), 3 $^{\circ}$C/min to 234 $^{\circ}$C, 10 $^{\circ}$C/min to 265 $^{\circ}$C (3 min)</p> <p>Injector: 280 $^{\circ}$C</p> <p>Transfer line temp: 300 $^{\circ}$C</p> <p>Carrier: Helium, 1.0 mL/min</p> <p>Split ratio: 15/1</p> <p>The retention times of the parent compound and its <i>bis</i>-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.</p> <p>Parent (5.2 min): 288 (M+, 54), 273 (8), 270 (11), 246 (32), 228 (50), 147 (100), 124 (97) <i>m/z</i></p> <p><i>Bis</i> TMS (10.7 min): 432 (M+, 100), 417 (8), 327 (6), 301 (3), 208 (7), 73 (58) <i>m/z</i></p> <p>The <i>bis</i>-TMS derivative co-elutes with a comparison sample of silylated <i>epi</i>-testosterone and the two materials give matching mass spectra.</p>
TLC:	<p>Conditions: Kieselgel 60F₂₅₄. Chloroform/ethyl acetate (80:20)</p> <p>Single spot observed, R_f = 0.2</p>
IR:	<p>Instrument: FT-IR, Biorad WIN FTS40</p> <p>Range: 4000-400 cm^{-1}, KBr pellet</p> <p>Peaks: 3420, 1654, 1610, 1380, 1229, 1190, 872 cm^{-1}</p>
¹ H NMR:	<p>Instrument: Bruker DMX-500</p> <p>Field strength: 500 MHz</p> <p>Solvent: CDCl₃ (7.26 ppm)</p> <p>Key spectral data: δ 0.69 (3H, s), 1.17 (3H, s), 3.74 (1H, d), 5.71 (1H, s) ppm</p>
¹³ C NMR:	<p>Instrument: Bruker DMX-500</p> <p>Field strength: 126 MHz</p> <p>Solvent: CDCl₃ (77.16 ppm)</p> <p>Spectral data: δ 16.8, 17.3, 20.5, 24.5, 31.1, 32.2, 32.3, 32.8, 33.8, 35.6, 35.8, 38.6, 45.0, 48.1, 53.5, 79.5, 123.7, 171.2, 199.4 ppm</p>
Melting point:	216-218 $^{\circ}$ C
Microanalysis:	<p>Found: C = 78.9%; H = 9.6% (November 2000)</p> <p>Calculated: C = 79.1%; H = 9.8% (Calculated for C₁₉H₂₈O₂)</p>