Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

CIPM MRA

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

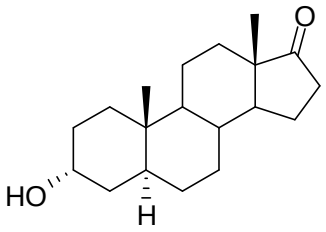
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D550c: Androsterone

Report ID: D550c.2019.01 (Bottled 180307)

Chemical Formula: $C_{19}H_{30}O_2$

Molecular Weight: 290.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
09-S-04	53-41-8	99.1 ± 0.7%

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

IUPAC name: (3α,5α)-3-Hydroxyandrostan-17-one

Expiration of certification: The property values are valid till 15 July 2024, 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: Off white crystalline powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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% 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 ten 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.
9 August 2019.

This report supersedes any issued prior to 9 August 2019.

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: Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA's behalf, assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this document.

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 and 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 QNMR using a certified internal standard of dimethyl sulfone and elemental microanalysis.

GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1/DB-17, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 240 °C (20 min), 20 °C/min to 280 °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 = 99.1%, s = 0.09% (7 sub samples in duplicate, July 2019)
GC-FID:	Instrument:	Varian CP-3800
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (10 sub samples in duplicate, August 2009)
GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (0.5 min), 20 °C/min to 230 °C (10 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 = 99.8%, s = 0.02% (9 sub samples in duplicate, August 2009)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	150 °C (0.5 min), 10 °C/min to 240 °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 = 99.7%, s = 0.01% (5 sub samples in duplicate, December 2010)
	Re-analysis:	Mean = 99.7%, s = 0.01% (6 sub samples in duplicate, September 2014)
GC-FID:	Instrument:	Agilent 7890
	Column:	HP-1MS, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	150 °C (0.5 min), 10 °C/min to 240 °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 = 99.7%, s = 0.03% (5 sub samples in duplicate, December 2011)
Thermogravimetric analysis:		Initial volatile content < 0.1% and non volatile residue < 0.2 % mass fraction (July 2009)
Karl Fischer analysis:		Moisture content < 0.1 % mass fraction (August 2009, December 2010, December 2011 and September 2014) Moisture content 0.3 % mass fraction (July 2019)

QNMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Internal standard:	Dimethyl sulfone (100% mass fraction)
	Initial analysis:	Mean = 99.4%, s = 0.14% (5 sub samples in duplicate, November 2009)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973N
	Column:	VF-1MS, 14.9 m × 0.25 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (1 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention time of the parent compound derivative is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (7.2 min):	290 (M+, 100), 275 (20), 272 (19), 257 (26), 246 (29), 239 (19), 215 (18), 201 (17), 165 (12), 147 (22), 124 (16), 107 (38), 93 (28), 79 (30), 67 (28), 55 (23) *m/z*
TLC:	Conditions:	Kieselgel 60F254. Hexane/ethyl acetate (4/1) Single spot observed, Rf = 0.27. Visualisation with vanillin
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3526, 2925, 2882, 2850, 1718, 1448, 1405, 1242, 1063, 1001 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance 400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.79 (3H, s), 0.76-0.86 (1H, m), 0.84 (3H, s), 1.00 (1H, m), 1.17-1.81 (18H, m), 1.90 (1H, m), 2.05 (1H, ddd, *J* = 9.1, 9.1, 19.2 Hz), 2.42 (1H, ddd, *J* = 0.7, 8.9, 19.2 Hz), 4.03 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	101 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 11.1, 13.8, 20.0, 21.7, 28.2, 29.0, 30.8, 31.5, 32.1, 35.0, 35.7 35.8, 36.2, 39.1, 47.8 51.4, 54.4, 66.3, 221.4 ppm
Melting point:		183-185 °C
Microanalysis:	Found:	C = 78.8%; H = 10.4% (August 2009)
	Calculated:	C = 78.6%; H = 10.4% (Calculated for $C_{19}H_{30}O_2$)