Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

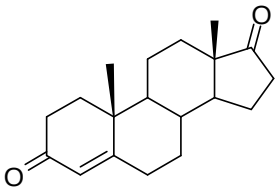
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA M955: Androstendione

Report ID: M955.2020.03 (Bottled 150527)

Chemical Formula: $C_{19}H_{26}O_2$

Molecular Weight: 286.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
04-S-02	**63-05-8**	**99.6 ± 0.3%**

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

Synonyms: 17β-Hydroxyandrost-4-en-3-one, Δ^4-Androsten-17β-ol-3-one, Androst-4-ene-17β-ol-3-one

Expiration of certification: The property values are valid till 16 June 2030, i.e. ten 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: White crystalline powder sourced from an external supplier, 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%.

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 1-2 mg sub samples 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.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
15 November 2022

This report supersedes any issued prior to 15 November 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 MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see http://www.bipm.org/kcdb/). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

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, 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 elemental microanalysis.

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.**

GC-FID:	Instrument:	Agilent 6890N or Agilent 7890	
	Column:	HP-1 or HP5, 30 m x 0.32 mm I.D. x 0.25 μm	
	Program:	220 °C (1 min), 4 °C/min to 280 °C, 10 °C/min to 300 °C (6 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.6%, s = 0.01% (10 sub samples in duplicate, April 2004)	
	Re- analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, July 2007)	
	Re- analysis:	Mean = 99.7%, s = 0.003% (5 sub samples in duplicate, July 2015)	
	Re- analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, June 2020)	
GC-FID:	Instrument:	Varian CP3800	
	Column:	VF-1MS, 30 m x 0.32 mm I.D. x 0.25 μm	
	Program:	220 °C (1 min), 4 °C/min to 280 °C, 10 °C/min to 300 °C (6 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative mass fraction of the main component:		
	Initial analysis:	Mean = 99.6%, s = 0.06% (5 sub samples in duplicate, April 2004)	
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (April 2004 and July 2006)	
Karl Fischer:		Moisture content < 0.2% mass fraction (June 2010, July 2015 and May 2020)	

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973	
Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min.	Split ratio: 20/1

Bis-TMS derivative:

Instrument:	HP5890/5971A	
Column:	BPX-5, 30 m x 0.22 mm I.D. x 0.25 μm	
Program:	220 °C, 10 °C/min to 300 °C (5 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 spectrum. The latter are reported in mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.0 min): 286 (M^+, 100), 244 (58), 201 (25), 187 (12), 173 (12), 148 (40), 124 (60), 107 (36), 91 (41), 79 (38), 67 (22), 55 (21), 41 (21) *m/z*

Bis-TMS: (9.6 min): 430 (M^+, 12), 208 (6), 207 (5), 75 (12), 73 (100) *m/z*

TLC: Conditions: Kieselgel 60F254. Dichloromethane / ethyl acetate (9:1)
Single spot observed, Rf = 0.3. Visualization with vanillin

IR:

Instrument:	Biorad WIN FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr
Peaks:	3447, 2951, 2921, 2847, 1734, 1663, 1615, 1452, 1227, 870 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX600
Field strength:	600 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.91 (3H, s), 0.98 (1H, ddd, *J* = 4.1, 11.0 & 12.3 Hz), 1.11 (1H, ddd, *J* = 4.3, 13.1 & 25.7 Hz), 1.21 (3H, s), 1.24-1.32 (2H, m), 1.45 (1H, ddd, *J* = 4.3, 13.3, 26.3 Hz), 1.56 (1H, m), 1.65-1.77 (3H, m), 1.86 (1H, ddd, *J* = 2.9, 3.8, 12.9 Hz), 1.94-2.00 (2H, m), 2.04 (1H, ddd, *J* = 3.2, 4.9, 13.3 Hz), 2.10 (1H, m), 2.29-2.51 (5H, m), 5.74 (1H, s) ppm

^{13}C NMR:

Instrument:	Bruker DMX500
Field strength:	126 MHz
Solvent:	$CDCl_3$ (77 ppm)
Spectral data:	δ 13.7, 17.3, 20.3, 21.7, 30.7, 31.2, 32.5, 33.9, 35.1, 35.6, 35.7, 38.6, 47.5, 50.8, 53.8, 124.1, 170.3, 199.3, 220.4 ppm

Melting point: 172-173 ºC from acetone

Microanalysis:

Found:	C = 79.8%; H = 9.1% (May 2004)
Calculated:	C = 79.7%; H = 9.2% (Calculated for $C_{19}H_{26}O_2$)