



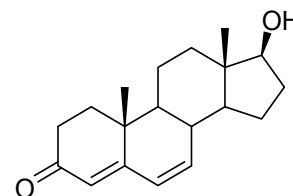
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S007: Androstan-4,6-dien-17 β -ol-3-one

Report ID: DS007.2017.02 (Ampouled 111201)

Chemical Formula: C₁₉H₂₆O₂

Molecular Weight: 286.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
11-S-04	2484-30-2	995 ± 16 µg

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

IUPAC name: (17 β)-17-Hydroxyandrosta-4,6-dien-3-one.

Expiration of certification: The property values are valid till 30 August 2022, 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 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 S007. This 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. methanol). This will transfer 995 ± 16 µg of anhydrous androstan-4,6-dien-17 β -ol-3-one. 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%.

Stability: This material has demonstrated stability over a minimum period of five 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 HPLC with UV detection 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.
11 February 2020

This report supersedes any issued prior to 11 February 2020

NATA logo notice: Accredited for compliance with ISO 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:

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
 Column: X-bridge C-18, 5 μm (4.6 mm x 150 mm)
 Column oven: 40 °C
 Mobile Phase: Methanol/MilliQ water (50:50)
 Flow rate: 1.2 mL/min
 Detector: Waters PDA 2998 operating at Max plot
 Relative mass fraction of the main component:
 Initial analysis: Mean = 99.7%, s = 0.02% (7 ampoules in duplicate, December 2011)
 Re-analysis: Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, December 2012)
 Re-analysis: Mean = 99.7%, s = 0.06% (5 ampoules in duplicate, November 2013)
 Re-analysis: Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, October 2014)
 Re-analysis: Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, August 2017)
 Detector: Waters PDA 2998 operating at 290 nm
 Relative mass fraction of the main component:
 Re-analysis: Mean = 99.9%, s = 0.004% (7 ampoules in duplicate, December 2011)

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 HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. 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.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
 Column: X-bridge C-18, 2.7 μm (4.6 mm x 150 mm)
 Column oven: 40 °C
 Mobile Phase: Methanol/MilliQ water (50:50)
 Flow rate: 1.2 mL/min
 Detector: Shimadzu PDASPD-M20A operating at Max plot
 Relative mass fraction of the main component:
 Initial analysis: Mean = 99.7%, s = 0.03% (10 sub samples in duplicate, August 2011)
 Detector: Shimadzu PDASPD-M20A operating at 290 nm
 Relative mass fraction of the main component:
 Initial analysis: Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, August 2011)
 Thermogravimetric analysis: Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (August 2011)
 Karl Fischer analysis: Moisture content 0.3% mass fraction (August 2011)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (2 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	<i>Bis</i> -TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS 1, 30 m x 0.25 mm I.D. x 0.25 μ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (2 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the parent compound and <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.	
	Parent (11.4 min):	286 (M ⁺ , 93), 253 (20), 227 (20), 161 (25), 151 (25), 136 (100), 129 (24), 115 (29), 107 (39), 91 (47), 79 (32), 55 (22) <i>m/z</i>
	<i>Bis</i> -TMS (11.7 min):	430 (M ⁺ , 100), 415 (20), 268 (10), 231 (11), 129 (10), 73 (88) <i>m/z</i>
LC/ESI -MS:	Instrument:	Micromass Quattro LC Micro
	Operation:	Positive ion mode, direct infusion at 20 μ L/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	28 V
	Peak:	309.3 (M+Na ⁺) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	None
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate (1/1) Single spot observed, R _f = 0.43. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm ⁻¹ , KBr powder
	Key peaks:	3398, 2952, 2930, 2857, 1653, 1647, 1617, 1559, 1457, 1360, 1287, 1066, 890, 668 cm ⁻¹
¹ H NMR:	Instrument:	Bruker Avance-400
	Field strength:	300 MHz
	Solvent:	DMSO-d ₆ (2.50 ppm)
	Key spectral data:	δ 0.72 (3H, s), 1.06 (3H, s), 0.97-1.13 (3H, m), 1.30-1.44 (7H, m), 1.51 (1H, m), 1.61 (1H, m), 1.72 (1H, m), 1.77 (1H, m), 1.84-1.96 (2H, m), 2.14-2.26 (2H, m), 2.54 (1H, m), 3.32 (1H, s), 3.47 (1H, m), 4.53 (1H, d, <i>J</i> = 4.8 Hz), 5.60 (1H, s), 6.11 (1H, bd, <i>J</i> = 9.7 Hz), 6.16 (1H, dd, <i>J</i> = 2.3, 9.8 Hz) ppm
	Methanol estimated at 0.2% mass fraction was observed in the ¹ H NMR	
¹³ C NMR:	Instrument:	Bruker Avance-400
	Field strength:	100 MHz
	Solvent:	DMSO-d ₆ (2.50 ppm)
	Spectral data:	δ 11.1, 16.0, 19.9, 22.6, 29.7, 33.4, 33.6, 35.6, 36.1, 37.2, 43.4, 47.8, 50.4, 79.5, 122.9, 127.5, 140.7, 163.2, 198.0 ppm
Melting point:	202-204 $^{\circ}$ C	
Microanalysis:	Found:	C = 64.1%; H = 4.2%; N = 5.6% (September, 2011)
	Calculated:	C = 64.1%; H = 4.2%; N = 5.6% (Calculated for C ₁₉ H ₂₆ O ₂)