Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

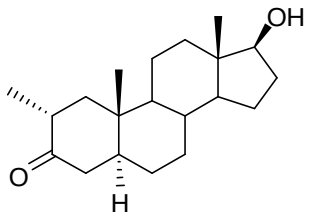
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S026b: Drostanolone

Report ID: S026b.2023.02 (Ampouled 200402)

Chemical Formula: $C_{20}H_{32}O_2$

Molecular Weight: 304.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
19-S-06	58-19-5	1002 ± 20 µg

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

IUPAC name: (2α,5α,17β)-17-Hydroxy-2-methylandrostan-3-one.

Expiration of certification: The property values are valid till 10 March 2026, 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 S026b. 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. chloroform). This will transfer 1002 ± 20 µg of anhydrous drostanolone. 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%.

Stability: This material has demonstrated stability over a minimum period of three 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 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 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.
2 May 2023

This report supersedes any issued prior to 02 May 2023.

NATA **a**ccreditation No. 198 / Corporate Site No. 14214.

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, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 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% (7 sub ampoules in duplicate, April 2020)
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 ampoules in duplicate, April 2021)
	Re-analysis:	Mean = 99.9%, s = 0.00% (5 ampoules in duplicate, March 2022)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, March 2023)

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 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.

Supporting evidence is provided by elemental microanalysis.

This material has shown signs of decomposition when injected at elevated temperature (250 ºC) onto a GC column, affording an artifact impurity peak immediately tailing the main analyte. This effect has also been observed in drostanolone esters, and has tentatively been attributed to isomerization at C2.

GC-FID:	Instrument:	Agilent 7890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 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 = 99.8%, s = 0.02% (7 sub samples in duplicate, August 2019)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (August 2019)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (September 2019)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	DB-5MS, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention time of the parent compound is reported 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 (13.5 min):	304 (M^+, 74), 286 (12), 271 (13), 260 (14), 245 (100), 231 (12), 199 (16), 177 (31), 161 (13), 149 (19), 138 (26), 121 (28), 107 (29), 105 (23), 93 (35), 81 (38), 79 (36), 67 (37), 55 (40) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m × 0.25 mm I.D. × 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, hexane
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (7:3) Single spot observed, R_f = 0.3. Visualisation with vanillin.
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3467, 3410, 2926, 2848, 1716, 1700, 1453, 1378, 1336, 1193, 1134, 1050, 1028 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.71 (1H, m), 0.76 (3H, s), 0.87 (1H, m), 0.96 (1H, m), 1.00 (3H, d, *J* = 6.5 Hz), 1.07 (3H, s), 1.02-1.13 (2H, m), 1.25 (1H, m), 1.29-1.66 (9H, m), 1.70 (1H, dq, *J* = 13.0, 3.5 Hz), 1.81 (1H, ddd, *J* = 12.5, 3.7, 3.0 Hz), 2.02-2.09 (3H, m), 2.31 (1H, dt, *J* = 0.5, 14.0 Hz), 2.46 (1H, septet, *J* = 6.4 Hz), 3.63 (1H, t, *J* = 8.6 Hz) ppm
	Ethyl acetate estimated at 0.1% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 11.3, 12.6, 14.8, 21.3, 23.6, 28.8, 30.7, 31.5, 35.5, 36.7, 36.8, 41.3, 43.2, 44.9, 48.2, 48.8, 51.0, 54.2, 82.0, 213.3 ppm
Melting point:		158-159 °C
Microanalysis:	Found:	C = 78.9%; H = 10.6% (September, 2019)
	Calculated:	C = 79.2%; H = 10.6% (Calculated for $C_{20}H_{32}O_2$)