Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

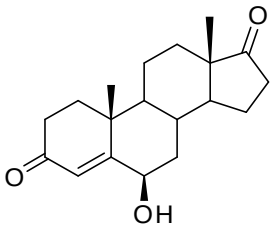
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S058: 6β-Hydroxyandrostenedione

Report ID: S058.2024.01 (Ampouled 240711)

Chemical Formula: $C_{19}H_{26}O_3$

Molecular Weight: 302.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
24-S-04	**63-00-3**	**983 ± 14 µg**

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

IUPAC name: (6β)-6-Hydroxyandrost-4-ene-3,17-dione.

Expiration of certification: The property values are valid till 19 July 2027, three years from the date of 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: White solid prepared by synthesis and certified for identity and purity by NMI Australia. The analyte is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon, intended for single use to prepare a standard solution.

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. acetonitrile). This will transfer 983 ± 14 µg of anhydrous 6β-hydroxyandrostenedione. 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 all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: In the absence of long-term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years. The measurement uncertainty at the 95% confidence interval also includes a stability component determined from accelerated stability trials conducted at 40 ºC and 75% humidity for 14 days.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on eight randomly selected 1-2 mg sub samples of the candidate 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.
22 October 2024

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (30:70 v/v)
	Flow rate:	1.0 mL/min
	Detector:	RS Diode Array Detector operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.01% (7 ampoules in duplicate, July 2024)

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, and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC-UV detection, 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.

HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (30:70 v/v)
	Flow rate:	1.0 mL/min
	Detector:	RS Diode Array Detector operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.004% (8 sub samples in duplicate, May 2024)
Thermogravimetric analysis:		Volatile content 0.2% and non-volatile residue 0.8% mass fraction (May 2024)
Karl Fischer analysis:		Moisture content 0.5% mass fraction (May 2024)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 8890/5977B
	Column:	HP-5MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	160 °C (1 min), 10 °C/min to 220 °C (7 min), 20 °C/min to 300 °C (7 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	The retention time of the parent compound is reported 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 (19.3 min):	302 (M^+, 100), 287 (50), 273 (22), 259 (6), 231 (8), 152 (40), 131 (15), 123 (22), 110 (23), 105 (24), 93 (28), 79 (37), 67 (29), 55 (25) *m/z*
ESI-MS:	Instrument:	Shimadzu LC-TQ-MS 8045
	Operation:	Direct infusion at 10 μL/min
	Ionisation mode:	Electrospray negative ion
	Interface voltage:	3.0 kV
	Peak:	301 ($M-H^+$) *m/z*
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.94 (3H, s), 0.97 (1H, ddd, *J* = 4.1, 10.9, 12.2 Hz)), 1.24-1.34 (3H, m), 1.40 (3H, s), 1.51 (1H, ddd, *J* = 4.1, 13.1, 26.1 Hz), 1.62 (1H, m), 1.65-1.75 (2H, m), 1.87 (1H, ddd, *J* = 2.8, 3.9, 13.1 Hz), 1.95-2.20 (6H, m), 2.38 (1H, dm, J = 17.1 Hz), 2.45-2.54 (2H, m), 4.40 (1H, bs), 5.82 (1H, s) ppm
	Ethyl acetate 0.2% and dichloromethane 0.03% mass fraction was observed in the ^{1}H NMR (May 2024).	
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.16 ppm)
	Spectral data:	δ 13.9, 19.7, 20.4, 21.9, 29.6, 31.4, 34.3, 35.9, 37.2, 37.4, 38.2, 47.8, 51.0, 53.8, 72.9, 126.7, 167.9, 200.3, 220.6 ppm
Melting point:		195-196 ºC
Microanalysis:	Found:	C = 74.7%; H = 8.4% (June 2024)
	Calculated:	C = 75.5%; H = 8.7% (Calculated for $C_{19}H_{26}O_3$)
	Calculated:	C = 75.0%; H = 8.7% (Calculated for $C_{19}H_{26}O_3$, 0.5%, water and 0.2% ethyl acetate)