Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

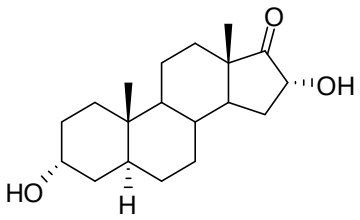
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D843: 16α-Hydroxyandrosterone

Report ID: D843.2019.01 (Ampouled 050707)

Chemical Formula: $C_{19}H_{30}O_3$

Molecular Weight: 306.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
03-S-09	14167-49-8	984 μg

IUPAC name: (3α,5α,16α)-3,16-Dihydroxyandrostan-17-one.

Expiration of certification: The property values are valid till 3 October 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 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 and is intended for a single use to prepare a standard solution containing D843. This material was prepared by sourced from an external supplier and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

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 984 μg of anhydrous 16α-hydroxyandrosterone.

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.

Stability: 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 five 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.
18 October 2019

This report supersedes any issued prior to 18 October 2019.

Characterisation Report:

HPLC:	Instrument:	Waters 1525 binary pump, W717 autosampler, Waters Alliance 2695 Separations module
	Column:	Alltima C-18 5 µm (4.6 mm ×150 mm)
	Mobile Phase:	Acetonitrile/Milli Q Water (42:58)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 or 2998 operating at 205 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (3 ampoules in duplicate, July 2005)
	Re-analysis:	Mean = 99.7%, s = 0.04% (5 ampoules in duplicate, August 2006)
	Re-analysis:	Mean = 99.8%, s = 0.03% (5 ampoules in duplicate, August 2009)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, July 2014)
	Re-analysis:	Mean = 99.8%, s = 0.03% (6 ampoules in duplicate, October 2019)
	Detector:	Waters ELSD 2420
	Initial analysis:	Mean = 99.98%, s = 0.01% (5 ampoules in duplicate, August 2009)

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 purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV/ELS detection, thermogravimetric 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 qualitative elemental microanalysis.

HPLC:	Instrument:	Waters 1525 binary pump, W717 autosampler
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/ Water (42:58)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at 205 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.03% (7 sub samples in duplicate, October 2003)
	Re-analysis:	Mean = 99.7%, s = 0.04% (5 sub samples in duplicate, August 2005)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction. (October 2003 & August 2006)

Spectroscopic and other characterisation data

GC-MS:	*Bis*-TMS derivative:	
	Instrument:	HP6890/5973
	Column:	Ultra 1, 17 m × 0.20 mm ID × 0.11 µm
	Program:	189 °C (0.2 min) 3 °C /min to 240 °C, 10 °C /min to 265 °C, 30 °C /min to 310 °C (2 min)
	Injector:	250 °C
	Transfer line temp:	300 °C
	Carrier:	Helium 1mL/min
	Split ratio:	14/1
	The retention time of the *bis*-TMS derivative is reported 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 intensity of the base peak.	
	11.5 min:	450 (M^+, 2), 435 (4), 306 (51), 216 (100), 201 (54), 117 (74), 106 (58), 73 (60) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/Ethyl acetate (1:1) Single spot observed, R_f = 0.17
IR:	Instrument:	FT-IR, Biorad FTS3000MX
	Range:	4000-400 cm^{-1}, KBr
	Peaks:	3517, 3348, 1739, 1447, 1247, 1150, 1014, 999, 929, 907, 617 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6
	Key spectral data:	δ 0.74 (3H, s), 0.82 (3H, s), 0.91 (1H, m), 1.86 (1H, m), 3.80 (1H, br s), 4.18 (2H, m), 5.30 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	DMSO-d_6
	Spectral data:	δ 11.5, 14.4, 19.7, 28.4, 29.0, 30.8, 31.9, 32.2, 34.8, 36.1, 36.1, 38.9, 47.4, 48.4, 54.4, 64.4, 70.5, 219.4 ppm
Melting point:		202-205 °C
Microanalysis:	Found:	C = 74.4%, H = 9.9% (August 2003)
	Calculated:	C = 74.5%, H = 9.9% (Calculated for $C_{19}H_{30}O_3$)