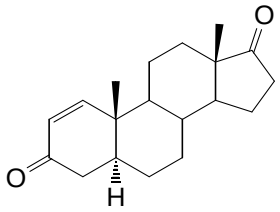
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D845.2017.01 (Ampouled 100330)
This batch of ampoules was prepared from the bulk material on 30th March 2010.

Compound Name: **1-Androstendione**
Collection Number: D845
Chemical Formula: $C_{19}H_{26}O_2$
CAS Registry Number: 571-40-4
Structure:



Description: White powder
Batch Number: 03-S-23
Molecular Weight: 286.4
Batch production completed: February 2004

Synonyms: 5α-Androst-1-en-3,17-dione

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 D845. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methyl tert-butyl ether). This will transfer 981 ± 16 µg of anhydrous 1-Androstendione. The uncertainty is stated at the 95% coverage interval.

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm	
	Program:	210 °C (20 min), 20 °C/min to 300 °C (5 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.5%, s = 0.02% (7 ampoules in duplicate, April 2010)	
	Re-analysis:	Mean = 98.5%, s = 0.03% (5 ampoules in duplicate, May 2011)	
	Re-analysis:	Mean = 98.5%, s = 0.02% (5 ampoules in duplicate, March 2014)	
	Re-analysis:	Mean = 98.5%, s = 0.01% (5 ampoules in duplicate, February 2017)	

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The purity value was obtained from a combination of traditional analytical techniques by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	HP 6890	
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm	
	Program:	210 °C (20 min), 30 °C/min to 300 °C (5 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 30/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.3%, s = 0.02% (10 sub samples in duplicate, October 2003)	
Thermogravimetric analysis:		Volatiles content and non-volatile residue < 0.3% mass fraction (October 2003, February 2006, February 2010)	
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (February 2010)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP 5890/5971A	
Column:	BPX-5, 30 m x 0.25 mm I.D. x 0.25 μm	
Program:	220 °C (2 min), 10 °C /min to 300 °C (5 min)	
Injector:	280 °C	Transfer line temp: 310 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

Bis-TMS derivative:

Instrument:	Agilent 6890/5973	
Column:	Ultra 1, 17m x 0.2mm I.D.x 0.11μm	
Program:	189 °C (0.2 min), 3 °C /min to 240 °C, 10 °C /min to 265, 30 °C/min to 310 °C (3 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 14:1

The retention times of the parent compound and *bis*-TMS derivative are 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 (10.9 min): 286 (M^+, 18), 244 (33), 202 (17), 147 (17), 122 (100), 109 (41), 91(32), 79 (40) m/z

Bis-TMS (10.3 min): 430 (29, M^+), 415 (94), 223 (14), 221 (15), 194 (29), 179 (17), 147 (16), 73 (100) m/z

Note: Treatment with MSTFA/TMSI or neat MSTFA results in the formation of artefact peaks in addition to the derivatised material.

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (3:1)
Single spot observed, R_f = 0.54. Visualization with UV light (254 nm)

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	2930, 2854, 1739, 1674, 1467, 1377, 1251, 1049, 780 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-600	
Field strength:	600 MHz	Solvent: $CDCl_3$ (7.3 ppm)
Key spectral data:	δ 0.90 (3H, s), 1.03 (3H, s), 2.24 (1H, dd), 2.38 (1H, dd), 2.46 (1H, dd,), 5.86 (1H, d), 7.12 (1H, d) ppm	

^{13}C NMR:

Instrument:	Bruker DMX-600	
Field strength:	150.9 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.0, 13.9, 20.5, 21.7, 27.3, 30.1, 31.4, 35.2, 35.7, 39.0, 40.9, 44.2, 47.8, 50.1, 51.2, 127.6, 157.7, 199.8, 220.5 ppm	

Melting point: 140-143 °C

Microanalysis: Found: C = 79.4%; H = 9.2% (December 2003)
Calc: C = 79.7%; H = 9.2% (Calculated for $C_{19}H_{26}O_2$)

Expiration of certification
The property values are valid till 14th February 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.

The long-term stability of the compound in solution has not been examined.

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 annual stability trials.

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.

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.

Intended use
For ***in vitro*** laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R Davies
Team Leader,
Chemical Reference Materials, NMI
Dated: 1 March, 2017.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 1st March 2017.