Australian Government

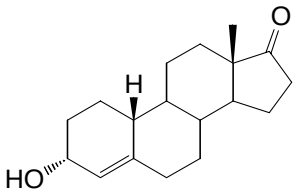
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D873.2014.01

Compound Name: **3α-Hydroxyestrenone**
Collection Number: D873
Chemical Formula: $C_{18}H_{26}O_2$
CAS Registry Number: 65336-99-4
Description: White powder
Batch Number: 03-S-15
Molecular Weight: 274.4
Release date: 21st November 2005

Structure:



Synonyms: 3α-Hydroxy-4-estren-17-one

Purity (mass fraction): 96.2 ± 3.6% (95% coverage interval)

Purity estimate obtained from Quantitative NMR using certified dimethyl sulfone as the internal standard. Impurity estimates by HPLC and TGA are also provided.

Note: This material shows signs of decomposition when injected onto a silanised glass GC liner and subjected to temperatures between 130-250 °C.

QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: $CDCl_3$
	Internal standard:	Dimethyl sulfone (100% m/m)
	Initial analysis:	Mean = 95.6%, s = 0.9% (4 sub samples, January 2007)
	Re-analysis:	Mean = 96.9%, s = 0.5% (3 sub samples, January 2008)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: CD_3OD
	Internal standard:	Dimethyl sulfone (100% m/m)
	Initial analysis:	Mean = 96.1%, s = 0.4% (5 sub samples, January 2011)
	Re-analysis:	Mean = 97.3%, s = 0.4% (2 sub samples in duplicate, February 2014)
HPLC:	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (50:50)
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, September 2005)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, October 2006)

Thermogravimetric analysis: Volatile content < 0.1% and non-volatile content < 0.2% mass fraction (November 2006)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973	
Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium 1.0 mL/min	Split ratio: 20/1

Bis-TMS Derivative:

Instrument:	Agilent 6890/5973	
Column:	Ultra 1, 17 m x 0.2 mm I.D. x 0.11μm	
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, 1.0 mL/min	Split ratio: 14/1

The retention times of the parent compound and the *bis*-TMS derivative are reported along 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 (7.8 min): 274 (M^+, 61), 256 (100), 216 (31), 199 (29), 149 (27), 131 (27), 105 (40), 91 (90), 79 (51), 77 (36), 67 (32), 55 (28), 41 (29) m/z

Bis-TMS (8.4 min): 418 (M^+, 45), 403 (60), 328 (27), 313 (33), 261 (6), 195 (10), 181 (15), 169 (22), 155 (11), 143 (12), 129 (10), 91 (21), 73 (100) m/z

TLC: Conditions: Kieselgel 60F_{254} 100% Ethyl acetate. Single spot observed, R_f = 0.51. Visualization with vanillin, H_2SO_4 spray

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3476, 2919, 2850, 1721, 1661, 1436, 1409, 1375, 1187, 1086, 1046, 954, 851 cm^{-1}

^{1}H NMR:

Instrument:	Gyro 300	
Field strength:	300 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.78 (1H, m), 0.91 (3H, s), 2.30 (1H, ddd, *J* = 2.6, 3.9, 14.0 Hz), 2.45 (1H, dd, *J* = 8.5, 19.0 Hz), 4.13 (1H, m), 5.58 (1H, d, *J* = 4.5 Hz), ppm	

^{13}C NMR:

Instrument:	Bruker Advance 300	
Field strength:	75.5 MHz	Solvent: $CDCl_3$ (77.16 ppm)
Spectral data:	δ 13.8, 21.7, 22.7, 25.8, 30.4, 30.4, 31.5, 35.0, 35.8, 40.3, 42.0, 47.9, 49.9, 50.4, 64.4, 122.1, 144.9, 221.0 ppm	

Microanalysis: Found: C = 78.9%; H = 9.5% (April 2005)
Calc: C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)

Melting point: Melting point not determined

Thermogravimetric analysis: Volatile content < 0.1% and non-volatile content < 0.2% mass fraction (November 2006)

Expiration of certification
The property values are valid till 20th February 2019, 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 sample bottles 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 been given a shelf life of three years from the date of re-certification. 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 HPLC with ELS detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% coverage 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: 28 February, 2014.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 28th February 2014.