Australian Government

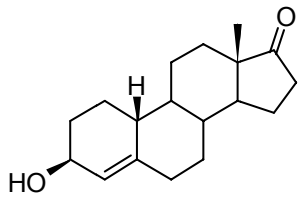
National Measurement Institute

ACCREDITED FOR TECHNICAL COMPETENCE

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D866.2015.01

Compound Name: **3β-Hydroxyestrenone**
Collection Number: D866
Chemical Formula: $C_{18}H_{26}O_2$
CAS Number: 15396-48-2
Structure:



Description: White powder
Batch Number: 03-S-13
Molecular Weight: 274.4
Release date: May 2004

Synonyms: 3β-Hydroxyestr-4-en-17-one

Purity (mass fraction): 93% minimum

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities observed in the ^{1}H NMR and moisture by Karl Fischer analysis. Supporting evidence is provided by elemental microanalysis.

Warning: This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column oven	40 °C
	Mobile Phase:	MilliQ water/Acetonitrile (A/B) 0-6 min 30% B, 6-18 min 30-50% B, 18-24 min 50% B, 24-25 min 50-30% B, 25-31 min 30% B
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating at Max Plot
	Relative peak area response of main component:	
	Initial analysis:	Mean = 96.8%, s = 0.3% (7 sub samples in duplicate, May 2007)
	Re-analysis:	Mean = 96.7%, s = 0.5% (5 sub samples in duplicate, June 2010)
	Re-analysis:	Mean = 97.4%, s = 0.3% (5 sub samples in duplicate, May 2015)
Thermogravimetric analysis:		Volatile content 0.6% and non-volatile content < 0.2% mass fraction (May 2007)
Karl Fischer analysis:		Moisture content < 0.9% mass fraction (May 2010) Moisture content 0.5 % mass fraction (May 2015)

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP6890/5973	
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, 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 °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 time of the parent compound and the *bis*-TMS is reported along 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 base peak.

Parent (7.8 min): 274 (M^+, 73), 256 (100), 216 (35), 199 (31), 160 (28), 131 (29), 105 (44), 91 (100), 79 (56), 77 (40), 67 (37), 55 (32), 41 (35) m/z

Bis-TMS (9.6 min): 418 (M^+, 69), 403 (100), 328 (12), 313 (22), 207 (15), 181 (16), 169 (21), 155 (10), 143 (10), 129 (7), 91 (13), 73 (99) m/z

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/Ethyl acetate (4:1), Single spot observed, R_f = 0.26. Visualization with vanillin, H_2SO_4 spray.

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3486, 2922, 2857, 1726, 1446, 1371, 1184, 1084, 1046, 935 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-600	
Field strength:	600 MHz	Solvent: $CDCl_3$
Key spectral data:	δ 0.66 (1H, ddd, *J* = 4.0, 10.9, 21.7 Hz), 0.89 (3H, s), 0.98 (1H, ddd, *J* = 4.0, 12.7, 25.5 Hz), 1.38 (1H, ddd, *J* = 3.2, 11.2, 21.7 Hz), 2.28 (1H, dm, *J* = 13.8 Hz), 2.44 (1H, dd, J = 8.8, 19.3 Hz), 4.16 (1H, m), 5.41 (1H, s) ppm	

Impurities observed at δ 0.87 and 0.88 ppm have been estimated as contributing between 5 and 6% mass fraction of the total mass of this material.

^{13}C NMR:

Instrument:	Bruker Advance 300	
Field strength:	75.5 MHz	Solvent: $CDCl_3$
Spectral data:	δ 13.8, 21.7, 25.3, 25.8, 30.6, 31.4, 32.1, 34.8, 35.8, 40.4, 41.8, 47.8, 50.2, 50.4, 67.3, 124.7, 142.4, 221.1 ppm	

Melting point: 130-132 °C

Microanalysis: Found: C = 78.3%; H = 9.3% (April 2005)
Calc: C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)

Expiration of certification
The property values are valid till 7th May 2020, 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 demonstrated stability over a minimum period of three 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 UV detection on seven randomly selected 1-2 mg samples of the material. The material was judged to be inhomogeneous at this level of sampling as the variation in analysis results between samples was 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: 18 May, 2015.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 18th May 2015.