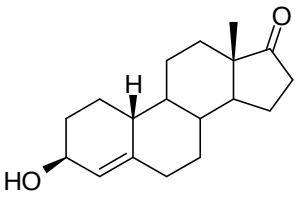
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D866: 3β-Hydroxyestrenone

Report ID: D866.2020.01

Chemical Formula: $C_{18}H_{26}O_2$

Molecular Weight: 274.4 g/mol



Property value

Batch No.	CAS No.	Purity by HPLC
03-S-13	15396-48-2	97.2%

IUPAC name: 3β-Hydroxyestr-4-en-17-one.

Expiration of certification: The property values are valid till 22 May 2025, 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.

Description: White powder sourced from an external supplier and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

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

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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: This material has demonstrated stability over a minimum period of five years. 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 seven randomly selected 1-2 mg sub samples 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 a 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 June 2020

This report supersedes any issued prior to 22 June 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. Impurities of related structure were assessed by HPLC with UV detection. The purity value was obtained by HPLC with UV detection.

Supporting evidence is provided by thermogravimetric analysis, Karl Fischer analysis, ^{1}H NMR spectroscopy and elemental microanalysis.

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 of the 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)
	Re-analysis:	Mean = 97.9%, s = 0.3% (5 sub samples in duplicate, May 2020)
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 times of the parent compound and the *bis*-TMS are 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)
	Calculated:	C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)