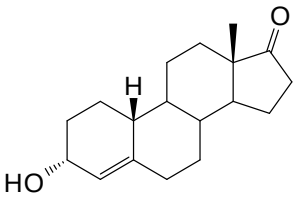
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D873: 3α-Hydroxyestrenone

Report ID: D873.2019.03

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

Molecular Weight: 274.4 g/mol



Property value

Batch No.	CAS No.	Purity (mass fraction)
03-S-15	65336-99-4	96.9 ± 2.3%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (3α)-3-Hydroxyestr-4-en-17-one.

Expiration of certification: The property values are valid till 26 June 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 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 prepared by synthesis, 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 measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 ELS detection on ten 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.
15 November 2022

This report supersedes any issued prior to 15 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by quantitative NMR and elemental microanalysis.

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

GC-FID:	Instrument:	Varian CP-3800
	Column:	DB-17, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	200 °C (1 min), 2 °C/min to 250 °C, 20 °C/min to 280 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 93.9%, s = 0.2% (7 sub samples in duplicate, June 2019)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$
	Internal standard:	Dimethyl sulfone (100% mass fraction)
	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% mass fraction)
	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 of the 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)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (June 2019)
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 × 0.25 mm I.D. × 0.30 µm
	Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium 1.0 mL/min
	Bis-TMS Derivative:	
	Instrument:	Agilent 6890/5973
	Column:	Ultra 1, 17 m × 0.2 mm I.D. × 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
	Split ratio:	14/1
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	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. Visualisation 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)
	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 Avance 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
Melting point:		Melting point not determined
Microanalysis:	Found:	C = 78.9%; H = 9.5% (April 2005)
	Calculated:	C = 78.8%; H = 9.6% (Calculated for $C_{18}H_{26}O_2$)