Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

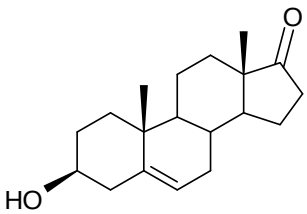
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D796b: Dehydroepiandrosterone

Report ID: D796b.2024.01

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
08-S-12	**53-43-0**	**99.7 ± 0.4%**

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

IUPAC name: (3β)-3-Hydroxyandrost-5-en-17-one

Expiration of certification: The property values are valid till 29 May 2029, 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 certified reference material is suitable for use as a primary calibrator.

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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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 GC-FID 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.
4 June 2024

This report supersedes any issued prior to 04 June 2024.

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

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see http://www.bipm.org/kcdb/). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

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 certified 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 (qNMR), qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis. The purity value by quantitative nuclear magnetic resonance (qNMR) was obtained using the one-proton multiplet at 5.50 ppm measured against a certified internal standard of maleic acid.

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 240 °C (9 min); 40 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (7 sub samples in duplicate, October 2008)
GC-FID:	Instrument:	Agilent 6890 or 7890
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 μm
	Program:	200 °C (20 min), 15 °C/min to 260 °C (1 min); 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.01% (5 sub samples in duplicate, October 2010)
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, August 2014)
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, May 2017)
	Re-analysis:	Mean = 99.7%, s = 0.07% (5 sub samples in duplicate, April 2020
	Re-analysis:	Mean = 99.8%, s = 0.14% (7 sub samples in duplicate, May 2024)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 220 °C (8.33 min); 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, October 2009)
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, October 2011)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (October 2008, October 2009, November 2010, October 2011, July 2014, June 2017, May 2024) Moisture content 0.2% mass fraction (May 2020)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (October 2008 and May 2024)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	AcOH-d_4 (2.05 ppm)
	Internal standard:	Maleic acid (99.9% mass fraction)
	Initial analysis:	Mean (5.5 ppm) = 99.7%, s = 0.12% (5 sub samples, July 2024)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	Bis-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μm
	Program:	180 °C, 3 °C /min to 240 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C
	Injector:	260 °C
	Split ratio:	30/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times of the parent compound and *bis*-TMS derivative are reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (10.2 min):	288 (M^+, 100), 270 (69), 255 (89), 237 (21), 213 (34), 203 (55), 177 (39), 159 (40), 145 (45), 133 (28), 119 (42), 105 (68), 97 (36), 91 (72), 79 (52), 55 (32) *m/z*
	Bis-TMS (10.1 min):	432 (M^+, 100), 417 (70), 327 (32), 303 (14), 169 (19), 129 (22), 73 (53) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4:1) Single spot observed, R_f = 0.3. Visualization with vanillin
IR:	Instrument:	Biorad WIN FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3503, 3461, 2935, 2897, 1731, 1456, 1435, 1371, 1298, 1247, 1065, 1028, 801 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.88 (3H, s), 1.00 (1H, m), 1.03 (3H, s), 1.10 (1H, m), 1.25-1.31 (2H, m), 1.45-1.57 (3H, m), 1.61-1.70 (4H, m, overlapping br s), 1.83-1.87 (3H, m), 1.95 (1H, m), 2.05-2.14 (2H, m), 2.24 (1H, m), 2.32 (1H, m), 2.45 (1H, dd, *J* = 8.7, 19.4 Hz), 3.53 (1H, m), 5.38 (1H, d, *J* = 5.3 Hz) ppm
^{13}C NMR:	Instrument:	Bruker GYRO 300
	Field strength:	75 MHz
	Solvent:	$CDCl_3$ (77 ppm)
	Spectral data:	δ 13.5, 19.4, 20.3, 21.8, 30.7, 31.3, 31.4, 31.5, 35.8, 36.6, 37.1, 42.2, 47.5, 50.2, 51.7, 71.5, 120.9, 141.0, 221.2 ppm
Melting point:		149-151 °C
Microanalysis:	Found:	C = 79.0%; H = 10.0% (September 2008)
	Calculated:	C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)