Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

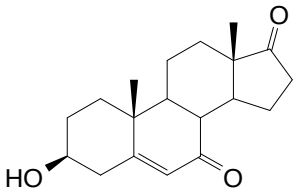
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D833: 7-Ketodehydroepiandrosterone

Report ID: D833.2023.01

Chemical Formula: $C_{19}H_{26}O_3$

Molecular Weight: 302.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
03-S-10	**566-19-8**	**98.9 ± 1.3%**

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-ene-7,17-dione.

Expiration of certification: The property values are valid till 1 May 2028, 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 25 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 approach all impurities are quantified as a mass fraction and subtracted from 100%.

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 UV detection on five 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.
17 May 2023

This report supersedes any issued prior to 17 May 2023.

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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, 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 qualitative elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler, Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltime C-18, 5 μm (4.6 mm x 150 mm); Ace Super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-5 min 40% B; 5-8 min 40-60% B; 8-15 min 60% B; 15-16 min 60-40% B; 16-20 min 40% B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA, Shimadzu PDA operating at 241 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (7 sub samples in duplicate, August 2003)
	Re-analysis:	Mean = 99.4%, s = 0.05% (5 sub samples in duplicate, June 2008)
	Re-analysis:	Mean = 99.1%, s = 0.03% (5 sub samples in duplicate, May 2013)
	Re-analysis:	Mean = 99.3%, s = 0.03% (5 sub samples in duplicate, May 2018)
	Re-analysis:	Mean = 99.0%, s = 0.01% (5 sub samples in duplicate, May 2023)
Karl Fischer analysis:		Moisture content is ≤ 0.2% mass fraction. (May 2008, 2013, May 2018, March 2023)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction. (October 2003 and August 2006).

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:	180 °C (1 min), 10 °C/min to 210 °C, 20 °C/min to 310 °C (6 min)
	Injector:	250 °C
	Split ratio:	15/1
	Transfer line temp:	310 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z* TMS derivative:
	Instrument:	HP 5890/5971A
	Column:	BPX-5, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 210 °C, 20 °C/min to 310 °C (6 min)
	Injector:	250 °C
	Split ratio:	15/1
	Transfer line temp:	310 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention times of the parent compound and 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 (10.5 min):	302 (M^+, 100), 269 (8), 205 (11), 187 (14), 161 (36), 91 (21) *m/z*
	Bis-TMS (12.5 min):	374 (M^+, 35), 207 (10), 163 (27), 129 (100), 73 (84) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (10:1) developed in ammonium molybdite/acidified ceric sulfate. Single spot observed, R_f = 0.2-0.3.
IR:	Instrument:	Biorad WIN FTS3000MX FTIR
	Range:	4000-400 cm^{-1},KBr powder
	Peaks:	3482, 2941, 2868, 1724, 1653, 1457, 1298, 1218, 1185, 1064 cm-1
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Key spectral data:	δ 0.89 (3H, s), 1.22 (3H, s), 2.55 (1H, ddd), 2.81 (1H, m), 3.69 (1H, m), 5.74 (1H, d) ppm
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	DMSO-d_6
	Spectral data:	δ 13.8, 17.4, 20.5, 24.2, 30.8, 31.4, 35.4, 36.3, 38.4, 42.1, 44.1, 45.4, 47.5, 49.8, 69.4, 125.1, 157.7, 200.8, 219.4 ppm
Melting point:		243-245 °C
Microanalysis:	Found:	C = 75.7%; H = 8.9%
	Calculated:	C = 75.5%; H = 8.7% (Calculated for $C_{19}H_{26}O_3$)