



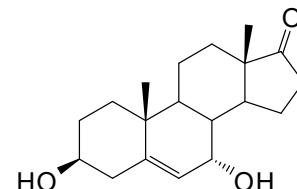
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D875: 7 $\alpha$ -HydroxyDHEA

Report ID: D875.2026.01 (Ampouled 170330)

Chemical Formula: C<sub>19</sub>H<sub>28</sub>O<sub>3</sub>

Molecular Weight: 304.4 g/mol



### Certified value

Batch No.	CAS No.	Purity (mass fraction)
03-S-11	53-00-9	937 ± 17 µg

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

**IUPAC name:** (3 $\beta$ ,7 $\alpha$ )-3,7-Dihydroxyandrost-5-en-17-one.

**Expiration of certification:** The property values are valid till 5 May 2036, ten 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

**Description:** The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D875. This material was sourced from an external supplier and certified for identity and purity by NMI Australia.

**Intended use:** This certified reference material is suitable for use as a primary calibrator.

**Instructions for use:** Open the ampoule and carefully rinse the interior at least three times, each time with a minimum of 0.5 mL of a suitable organic solvent (e.g. chloroform). This process will ensure the transfer of the stated mass per ampoule of anhydrous 7 $\alpha$ -hydroxydehydroepiandrosterone. The mass of analyte contained in each ampoule has been determined based on the assigned purity of the bulk material and the concentration of that bulk material in the stock solution used during ampoule preparation.

**Recommended storage:** When not in use, this material should be stored at or below 4 °C 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 ten 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 ampoules 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 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.  
2 June 2026

This report supersedes any issued prior to 2 June 2026.

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.

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## Characterisation Report:

GC-FID: Instrument: Agilent 6890 or Agilent 7890  
 Column: HP-1, 30 m  $\times$  0.32 mm I.D.  $\times$  0.25  $\mu$ m  
 Program: 180  $^{\circ}$ C (1 min), 30  $^{\circ}$ C/min to 250  $^{\circ}$ C (10 min), 30  $^{\circ}$ C/min to 300  $^{\circ}$ C (3 min)  
 Injector: 200 - 250  $^{\circ}$ C Detector Temp: 320  $^{\circ}$ C  
 Carrier: Helium Split ratio: 20/1

Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.1%, s = 0.005% (7 ampoules in duplicate, March 2017)  
 Re-analysis: Mean = 98.8%, s = 0.02% (5 ampoules in duplicate, May 2018)  
 Re-analysis: Mean = 99.1%, s = 0.03% (5 ampoules in duplicate, May 2019)  
 Re-analysis: Mean = 99.1%, s = 0.02% (5 ampoules in duplicate, June 2022)  
 Re-analysis: Mean = 98.9%, s = 0.02% (5 ampoules in duplicate, June 2026)

### The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

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

Supporting evidence is provided by elemental microanalysis.

GC-FID: Instrument: Agilent 5890 or Agilent 6890  
 Column: ZB-1or HP-1, 30 m  $\times$  0.32 mm I.D.  $\times$  0.25  $\mu$ m  
 Program: 180  $^{\circ}$ C (1 min), 40  $^{\circ}$ C/min to 250  $^{\circ}$ C (10 min), 40  $^{\circ}$ C/min to 300  $^{\circ}$ C (2 min)  
 Injector: 250  $^{\circ}$ C Detector Temp: 320  $^{\circ}$ C  
 Carrier: Helium Split ratio: 20/1

Relative mass fraction of the main component:  
 Initial analysis: Mean = 98.5%, s = 0.05% (7 sub samples in duplicate, May 2004)  
 Re-analysis: Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, October 2007)  
 Re-analysis: Mean = 98.6%, s = 0.08% (5 sub samples in duplicate, March 2009)  
 Re-analysis: Mean = 99.0%, s = 0.05% (5 sub samples in duplicate, February 2012)  
 Re-analysis: Mean = 99.1%, s = 0.04% (6 sub samples in duplicate, March 2017)

Thermogravimetric analysis: Volatile content 2.1% and non volatile residue < 0.2% mass fraction (June 2004 and October 2005)  
 Volatile content 1.9% and non-volatile residue < 0.2% mass fraction (October 2007)

Karl Fischer analysis: Moisture content 2.7% mass fraction (February 2007)  
 Moisture content 3.2% mass fraction (March 2009)  
 Moisture content 2.6% mass fraction (February 2012)  
 Moisture content 5.8% mass fraction (December 2016)

## Spectroscopic and other characterisation data

GC-MS: Parent compound:  
Instrument: Agilent 6890/5973  
Column: ZB-5, 30 m x 0.25 mm I.D. x 0.30  $\mu$ m  
Program: 220  $^{\circ}$ C (1 min), 10  $^{\circ}$ C/min to 300  $^{\circ}$ C (5 min)  
Injector: 250  $^{\circ}$ C Transfer line temp: 280  $^{\circ}$ C  
Carrier: Helium, 1.0 mL/min Split ratio: 20/1

*Tris*-TMS derivative:  
Instrument: Agilent 6890/5973  
Column: HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11  $\mu$ m  
Program: 189  $^{\circ}$ C (0.2 min), 3  $^{\circ}$ C/min to 240  $^{\circ}$ C, 10  $^{\circ}$ C/min to 265  $^{\circ}$ C,  
30  $^{\circ}$ C/min to 310  $^{\circ}$ C (2 min)  
Injector: 250  $^{\circ}$ C Transfer line temp: 300  $^{\circ}$ C  
Carrier: Helium, 1.0 mL/min Split ratio: 14/1

The retention times of the parent compound and *tris*-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 (8.4 min): 304 ( $M^+$ , 4), 286 (100), 271 (10), 145 (11), 143 (11), 119 (11), 105 (12), 91 (16), 79 (11), 55 (9) *m/z*

*Tris*-TMS(11.2 min): 520 (5), 430 (35), 415 (49), 325 (33), 250 (6), 235 (8), 195 (8), 181 (17), 169 (65), 129 (12), 73 (100) *m/z*

TLC: Conditions: Kieselgel 60F<sub>254</sub>. 100% Ethyl acetate  
Single spot observed,  $R_f$  = 0.2. Visualisation with vanillin, H<sub>2</sub>SO<sub>4</sub> spray

IR: Instrument: Biorad FTS3000MX FT-IR  
Range: 4000-400  $\text{cm}^{-1}$ , KBr powder  
Peaks: 3430, 3303, 2930, 2860, 1724, 1456, 1438, 1376, 1059, 1029, 1012, 953  $\text{cm}^{-1}$

<sup>1</sup>H NMR: Instrument: Bruker Gyro-300  
Field strength: 300 MHz  
Solvent: CDCl<sub>3</sub> (7.26 ppm)  
Spectral data:  $\delta$  0.89 (3H, s), 1.02 (3H, s), 3.58 (1H, m), 3.97 (1H, m), 5.64 (1H, dd,  $J$  = 1.9, 5.3 Hz) ppm

<sup>13</sup>C NMR: Instrument: Bruker Advance-300  
Field strength: 75.5 MHz  
Solvent: CDCl<sub>3</sub> (77.2 ppm)  
Spectral data:  $\delta$  13.3, 18.3, 20.1, 21.9, 31.1, 31.3, 35.8, 37.0, 37.2, 37.5, 41.9, 42.7, 45.0, 47.1, 64.3, 71.2, 123.6, 146.6, 221.0 ppm

Melting point: 175-177  $^{\circ}$ C

Microanalysis: Found: C = 73.1%, H = 9.8% (April 2009)  
Calculated: C = 75.0%, H = 9.3% (Calculated for C<sub>19</sub>H<sub>28</sub>O<sub>3</sub>)