Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

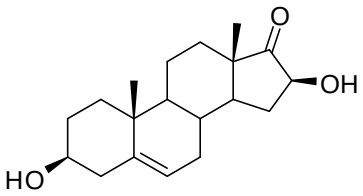
# REFERENCE MATERIAL PRODUCT INFORMATION SHEET

## NMIA D844: 16β-HydroxyDHEA

Report ID: D844.2020.01

Chemical Formula: $C_{19}H_{28}O_3$

Molecular Weight: 304.4 g/mol




## Property value

| Batch No. | CAS No. | Purity estimate |
|---|---|---|
| 03-S-14 | 1159-68-8 | 99.3% |

**IUPAC name:** (3β,16β)-3,16-Dihydroxyandrost-5-en-17-one

**Expiration of certification:** The property values are valid till 12 August 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:** Off-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 three 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.
14 August 2020

This report supersedes any issued prior to 14 August 2020.

## 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 mass balance from a combination of traditional analytical techniques, including HPLC with UV detection. 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 thermogravimetric analysis, Karl Fischer analysis and $^{1}$H NMR spectroscopy and elemental microanalysis.

| | | |
|---|---|---|
| HPLC: | Instrument: | Waters Model 1525 pump, 717 plus autosampler |
| | Column: | Alltech Alltima C-18, 5µm (4.6 mm x 150 mm) |
| | Column oven: | Ambient |
| | Mobile Phase: | Acetonitrile/MilliQ water (35:65 v/v) |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Waters 2998 PDA operating at 200 nm |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.8%, s = 0.02% (7 sub samples in duplicate, February 2004) |
| | Re-analysis: | Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, August 2008) |
| HPLC: | Instrument: | Waters Model 1525 pump, 717 plus autosampler |
| | Column: | Alltech Alltima C-18, 5µm (4.6 mm x 150 mm) |
| | Column oven: | Ambient |
| | Mobile Phase: | Acetonitrile/MilliQ water (30:70 v/v) |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Waters 2998 PDA operating at 200 nm |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.9%, s = 0.003% (5 sub samples in duplicate, September 2011) |
| | Re-analysis: | Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, August 2014) |
| HPLC: | Instrument: | Waters Alliance 2695 Separations module |
| | Column: | Grace Alltima C-18, 5 µm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | Acetonitrile / MilliQ water (30:70 v/v), Gradient 0-12 min 30% MeCN, 12-15 min 30-80% MeCN, 15-18 min 80% MeCN, 18-19 min 80-30% MeCN, 19-25 min 30% MeCN |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Waters 2998 PDA operating at 200 nm |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, August 2020) |
| Karl Fischer analysis: | | Moisture content < 0.1% mass fraction (August 2008, September 2011, July 2014 and July 2020) |
| Thermogravimetric analysis: | | Volatile content 0.1% and non volatile residue < 0.2 %mass fraction (January 2004 and April 2005) |

## Spectroscopic and other characterisation data

| | | |
|---|---|---|
| GC-MS: | *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 |
| | Injector: | 250 °C |
| | Split ratio: | 14/1 |
| | Transfer line temp: | 300 °C |
| | Carrier: | Helium, 1.0 mL/min |
| | Scan range: | 50-550 *m/z* |
| | The retention time of the *bis*-TMS derivative is 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. | |
| | *Bis*-TMS (13.7 min): | 448 ($M^+$, 5), 433 (8), 304 (61), 214 (100), 199 (55), 175 (36), 129 (66), 73 (55) *m/z* |
| TLC: | Conditions: | Kieselgel 60$F_{254}$. Chloroform/ethyl acetate (4:1)<br>Single spot observed, $R_f$ = 0.2<br>Visualisation with vanillin,$H_2SO_4$ spray |
| IR: | Instrument: | BioRad FTS3000MX FT-IR |
| | Range: | 4000-400 $cm^{-1}$, KBr powder |
| | Peaks: | 3402, 3317, 2930, 1732, 1459, 1433, 1403, 1373, 1300, 1048, 963, 912 $cm^{-1}$ |
| $^1$H NMR: | Instrument: | Bruker DMX-500 |
| | Field strength: | 500 MHz |
| | Solvent: | DMSO $d_6$ (2.5 ppm) |
| | Spectral data: | δ 0.82 (3H, s), 0.95 (3H, s), 3.24 (1H, m), 3.76 (1H, m), 4.60 (1H, d), 5.27 (1H, bd), 5.61 (1H, d) ppm |
| $^{13}$C NMR: | Instrument: | Bruker Gyro-300 |
| | Field strength: | 75.5 MHz |
| | Solvent: | $CDCl_3$ (77.0 ppm) |
| | Spectral data: | δ 14.7, 19.4, 20.1, 30.6, 30.6, 31.0, 31.6, 31.7, 36.8, 37.1, 42.2, 46.1, 46.7, 50.5, 71.6, 75.4, 120.8, 141.1, 220.1 ppm |
| Melting point: | | 197-204 °C |
| Microanalysis: | Found: | C = 75.0%; H = 9.3% |
| | Calculated: | C = 75.0%; H = 9.3% (Calculated for $C_{19}H_{28}O_3$) |