NMIA D867: 6β-Hydroxyetiocholanolone

Report ID: D867.2019.03 (Ampouled 110120)

Chemical Formula: C19H30O3

Molecular Weight: 306.4g/mol

# Certified value

|  |  |  |
| --- | --- | --- |
| **Batch No.** | **CAS No.** | **Mass per ampoule** |
| **03-S-08** | **14357-02-9** | **991 ± 11 g** |

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

IUPAC name: (3α,5β,6β)-3,6-Dihydroxyandrostan-17-one.

Expiration of certification: The property values are valid till 25 March 2029, 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 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 D867. This material was prepared by sourced from an external supplier and certified for identity and purity by NMIA.

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 with a suitable organic solvent (e.g. chloroform). This will transfer 991 ± 11 g of anhydrous 6β-hydroxyetiocholanolone.

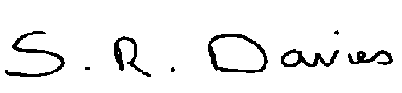
Recommended storage:When not in use, this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

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 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 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.



Dr Stephen R. Davies,

Team Leader,

Chemical Reference Materials, NMI.

12 April 2024

This report supersedes any issued prior to 12 April 2024.

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:

GC-FID: Instrument: Varian CP-3800 or Agilent 7890

Column: VF-1MS or HP-1MS, 30 m  0.32 mm I.D.  0.25 μm

Program: 180 C (1 min), 10 C/min to 240 C, 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 = 98.9%, s = 0.008% (7 ampoules in duplicate, March 2011)

Re-analysis: Mean = 98.8%, s = 0.03% (5 ampoules in duplicate, January 2014)

Re-analysis: Mean = 98.7%, s = 0.02% (5 ampoules in duplicate, November 2016)

Re-analysis: Mean = 98.8%, s = 0.01% (5 ampoules in duplicate, September 2019)

Re-analysis: Mean = 98.7%, s = 0.008% (5 ampoules in duplicate, March 2024)

**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 1H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - IORG) x (100 % - IVOL – INVR) Equation 1

IORG = Organic impurities of related structure, IVOL = volatile impurities, INVR = non-volatile residue.

Supporting evidence is provided by qualitative elemental microanalysis.

GC-FID: Instrument: Agilent 6890N

Column: HP-1, 30 m x 0.32 mm I.D. x 0.25 μm

Program: 180 C (1 min), 10 C/min to 240 C (10 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 = 98.9%, s = 0.10% (5 sub samples in duplicate, November 2010)

GC-FID: Instrument: Varian CP-3800

Column: VF-1, 30 m x 0.32 mm I.D. x 0.25 μm

Program: 180 C (1 min), 40 C/min to 250 C (10 min) 40 C/min to 300 C (2 min)

Injector: 250 C

Detector Temp: 320 C

Carrier: Helium

Split ratio: 20/1

Relative mass fraction of the main component:

Initial analysis: Mean = 98.9%, s = 0.10% (7 sub samples in duplicate, May 2004)

Re-analysis: Mean = 98.4%, s = 0.11% (5 sub samples in duplicate, October 2007)

Thermogravimetric analysis: Volatile content < 0.1% and non-volatile content < 0.2% mass fraction (October 2007)

Karl Fischer analysis: Moisture content < 0.4% mass fraction (October 2007 & November 2010)

**Spectroscopic and other characterisation data**

GC-MS: Parent compound:

Instrument: Agilent 6890/5973

Column: Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm

Program: 220 C (1 min), 10 C/min to 300 C (5 min)

Injector: 250 C

Transfer line temp: 280 C

Carrier: Helium, 1.0 mL/min

Split ratio: 20/1

*Tris*-TMS derivative:

Instrument: Agilent 6890/5973

Column: HP Ultra 1, 17 m  0.2 mm I.D.  0.11 μm

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

Transfer line temp: 300 C

Carrier: Helium, 1.0 mL/min

Split ratio: 14/1

The retention times of the parent compound and the *tris*-TMS derivative are reported along 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 (10.0 min): 306 (M+, 4), 288 (8), 273 (47), 233 (100), 199 (6), 159 (6), 95 (20),

79 (13), 67 (13), 55 (13), 41 (9) *m/z*

*Tris*-TMS (11.7 min): 522 (59), 507 (19), 417 (21), 377 (38), 327 (52), 169 (21), 147 (21),

73 (100) *m/z*

TLC: Conditions: Kieselgel 60F254. 100% Ethyl acetate, Single spot observed,

Rf = 0.21, Visualization with vanillin, H2SO4 spray

IR: Instrument: BioRad FTS3000MX FT-IR

Range: 4000-400 cm-1, KBr powder

Peaks: 3478, 3398, 2930, 1734, 1456, 1413, 1369, 1323, 1300, 1253, 1225, 1171, 1042, 1004, 921, 611 cm -1

1H NMR: Instrument: Gyro 300

Field strength: 300 MHz

Solvent: CD3OD (3.3 ppm)

Key spectral data:  0.88 (3H, s), 1.05 (1H, ddd, *J* = 3.0, 14.3, 14.3 Hz), 1.14 (3H, s),

2.45 (1H, dd, *J* = 8.7, 19.2 Hz), 3.62 (1H, m), 3.82 (1H, m), ppm

13C NMR: Instrument: Bruker Advance 300

Field strength: 75.5 MHz

Solvent: CD3OD (49 ppm)

Spectral data:  13.8, 19.9, 21.8, 25.5, 30.1, 30.5, 31.6, 33.5, 34.5, 35.8, 35.9, 36.3, 41.0

47.9, 48.8, 51.4, 71.1, 72.8, 221.1 ppm

Melting point: 221-223 °C

Microanalysis: Found: C = 74.5%, H = 9.6% (April 2005)

Calculated: C = 74.5%, H = 9.9% (Calculated for C19H30O3)

**Amendment record**

Original report ID: D867. 2011.01 (Ampouled 110120)

Date of issue: 21st March, 2011

* New batch of ampoules 110120
* Bulk analytical data carried over from D867.2010.01 report
* Given 3 years in report. 35 months in stability database (identification only).

Date of revision: 17th July 2012 (TD)

Revised report ID: D867.2011.02 (Ampouled 110120)

Revisions:

* Changed to the new address and contact details
* Updated NATA accreditation number to the current address

Date of revision: 16th January 2014

Revised report ID: D867.2013.01 (Ampouled 110120)

Revisions:

* GC-FID data updated, given 3 years expiry and CRM status.

Date of revision: 15th November 2016 (TD/MM)

Revised report ID: D867.2016.01 (Ampouled 110120)

Revisions:

* NATA logos moved in response to NATA audit March 2014.
* Updated GC data for 2016
* Purity statement remains unchanged but only update to new format 991 **±** 11 ug
* Five years shelf-life is given

Date of revision: 27 August 2019 (GJT/Approver)

Revised report ID: D867.2016.02

Revisions:

* Updated to the new report template.

Date of revision: 10 September 2019 (TD/MM)

Revised report ID: D867.2019.01 (Ampouled 110120)

Revisions:

* Corrected product release date in the footer
* Corrected the chemical formula for H & O
* Updated GC-FID data
* The stated mass in ampoule remains unchanged @ 991 µg ± 11 µg
* 5 years shelf-life is given

###### Date of revision: 27 July 2021 (TD)

###### Revised report ID: D867.2019.02 (Ampouled 110120)

Revisions:

* Updated the new departmental name
* Update the correct corporate site number

###### Date of revision: 15 November 2022 (TD)

###### Revised report ID: D867.2019.03 (Ampouled 110120)

Revisions:

* Update to the new template (change departmental name, NATA logo…)

###### Date of revision: 26 March 2024 (CW/MM)

###### Revised report ID: D867.2024.01 (Ampouled 110120)

Revisions:

* Routine stability check