



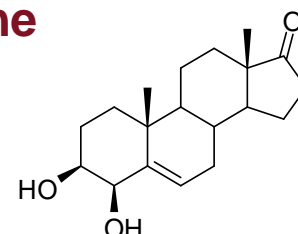
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D834: 4 β -Hydroxydehydroepiandrosterone

Report ID: D834.2016.02 (Ampouled 050505)

Chemical Formula: C₁₉H₂₈O₃

Molecular Weight: 304.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
03-S-13	63518-24-1	996 ± 6 µg

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

IUPAC name: (3 β ,4 β)-3,4-Dihydroxyandrost-5-en-17-one.

Expiration of certification: The property values are valid till 17 November 2021, 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D834. 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. methanol). This will transfer 996 ± 6 µg of anhydrous 4 β -hydroxydehydroepiandrosterone. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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 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 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.
28 April 2020

This report supersedes any issued prior to 21 April 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
 Column: Alltima C-18 5 µm (4.6 mm x 150 mm)
 Mobile Phase: A = MilliQ Water; B = Acetonitrile
 0-8 min 40% B; 8-9 min 40-60% B; 9-15 min 60% B; 15-16 min 60-40% B; 16-22 min 40% B.
 Flow Rate: 1.0 mL/min
 Detector: Waters PDA 996 operating at 201 nm [2005, 2006, 2007]/Waters 2998 PDA operating at 201 nm [2011, 2016]

Relative mass fraction of main component:

Initial analysis: Mean = 99.6%, s = 0.005% (3 ampoules in duplicate, May 2005)
 Re-analysis: Mean = 99.5%, s = 0.02% (3 ampoules in duplicate, May 2006)
 Re-analysis: Mean = 99.5%, s = 0.02% (5 ampoules in duplicate, May 2007)
 Re-analysis: Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, December 2011)
 Re-analysis: Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, November 2016)

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 HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ¹H 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_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by qualitative elemental microanalysis.

HPLC: Column: Alltima C-18 5 µm (4.6 mm x 150 mm)
 Mobile Phase: Acetonitrile/Water (40:60 v/v)
 Flow Rate: 1.0 mL/min
 Detector: UV at 201 nm

Relative mass fraction of the main component:

Initial analysis: Mean = 99.6%, s = 0.02% (7 sub samples in duplicate, July 2003)
 Re-analysis: Mean = 99.6%, s = 0.01% (5 sub samples in duplicate, May 2005)

Thermogravimetric analysis: Volatiles content and non-volatile residue < 0.3% total (October 2003 & May 2005)

Karl Fischer analysis: Moisture content < 0.1% mass fraction (July 2003)

Spectroscopic and other characterisation data

GC-MS: Instrument: HP 5890/5971A
 Column: BPX-5, 30 m \times 0.22 mm I.D. \times 0.25 μ m
 Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (5 min)
 Injector: 230 $^{\circ}$ C
 Transfer line temp: 320 $^{\circ}$ C
 Carrier: Helium
 Split ratio: 20/1

The retention time of the tris-TMS derivative is reported along 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.

13.8 min: 520 (M^+ , 1), 195 (6), 169 (16), 147 (17), 129 (13), 73 (100) m/z

TLC: Conditions: Kieselgel 60F₂₅₄. Hexanes/Ethyl acetate (1:1)
 Single spot observed, R_f = 0.25

IR: Instrument: Biorad FTS3000MX FT-IR
 Range: 4000-400 cm^{-1} , KBr powder
 Peaks: 3335, 2943, 1744, 1662, 1449, 1080, 1055, 967 cm^{-1}

¹H NMR: Instrument: Bruker DMX-600
 Field strength: 600 MHz Solvent: CD₃OD (3.31 ppm)
 Key spectral data: δ 0.94 (3H, s), 1.27 (3H, s), 2.50 (1H, dd), 3.50 (1H, ddd), 4.12 (1H, d), 5.71 (1H, dd) ppm

¹³C NMR: Instrument: Bruker DMX-300
 Field strength: 75 MHz Solvent: CDCl₃ (77.16 ppm)
 Spectral data: δ 13.5, 19.8, 20.9, 21.8, 25.2, 30.9, 31.3, 31.4, 35.8, 36.1, 36.9, 47.5, 50.3, 51.9, 72.3, 77.1, 127.7, 142.9, 221.0 ppm

Melting point: 197-200 $^{\circ}$ C

Microanalysis: Found: C = 74.9%; H = 9.5% (May 2003)
 Calculated: C = 74.9%; H = 9.3% (Calculated for C₁₉H₂₈O₃)