



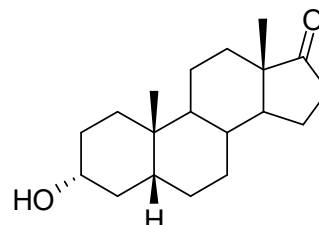
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D551c: Etiocholanolone

Report ID: D551c.2026.01 (Ampouled 260911)

Chemical Formula:  $C_{19}H_{30}O_2$

Molecular Weight: 290.4 g/mol



### Certified value

| Batch No. | CAS No. | Mass per ampoule         |
|-----------|---------|--------------------------|
| 10-S-07   | 53-42-9 | $995 \pm 15 \mu\text{g}$ |

The uncertainty has been calculated according to ISO 33405 and is stated at the 95% confidence limit ( $k = 2$ ).

**IUPAC name:** (3 $\alpha$ ,5 $\beta$ )-3-Hydroxyandrostane-17-one

**Expiration of certification:** The property values are valid till 14 September 2029, three years from the date of 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. The material will be re-tested periodically to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

**Description:** Off white solid was sourced from an external supplier and certified for identity and purity by NMI Australia. The analyte is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon, intended for a single use to prepare a standard solution containing D551c.

**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., *tert*-butyl methyl ether). This process will ensure the transfer of the stated mass per ampoule of anhydrous etiocholanolone. 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 all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

**Stability:** At the recommended storage conditions this material has demonstrated stability for a period of three years. The measurement uncertainty includes components for long term stability at the recommended storage conditions and accelerated stability trials conducted at 40 °C and 75% humidity for 14 days.

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.  
6 October 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 8890  
 Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 µm  
 Program: 215 °C (20 min), 20 °C/min to 300 °C (5 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.4%, s = 0.003% (7 ampoules in duplicate, September 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 <sup>1</sup>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 qualitative headspace GC-MS analysis of occluded solvents, quantitative nuclear magnetic resonance (qNMR) and elemental microanalysis.

GC-FID: Instrument: Agilent 6890 or 7890  
 Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 µm  
 Program: 215 °C (20 min), 20 °C/min to 300 °C (5 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.4%, s = 0.05% (10 sub samples in duplicate, July 2010)  
 Re-analysis: Mean = 99.3%, s = 0.09% (5 sub samples in duplicate, August 2012)  
 Re-analysis: Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, April 2015)

GC-FID: Instrument: Varian 3800  
 Column: HP-5, 30 m × 0.32 mm I.D. × 0.25 µm  
 Program: 215 °C (20 min), 20 °C/min to 300 °C (5 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.4%, s = 0.06% (10 sub samples in duplicate, July 2010)

GC-FID: Instrument: Varian 3800  
 Column: VF-1, 30 m × 0.32 mm I.D. × 0.25 µm  
 Program: 215 °C (20 min), 20 °C/min to 300 °C (5 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.4%, s = 0.05% (10 sub samples in duplicate, July 2010)

Karl Fischer analysis: Moisture content ≤ 0.1% mass fraction (August 2010, July 2012, April 2015 and July 2019)

Thermogravimetric analysis: Volatiles content 0.6% and non-volatile residue < 0.2% mass fraction (August 2010)

qNMR: Instrument: Bruker Avance-400  
 Field strength: 400 MHz  
 Solvent: CDCl<sub>3</sub> (7.26ppm)  
 Internal standard: Dimethyl terephthalate (100.0% mass fraction)  
 Initial analysis: Mean (2.4 ppm) = 98.6%, s = 0.8% (5 sub samples, August 2010)

## Spectroscopic and other characterisation data

|                                                                                                                                                                                                                       |                     |                                                                                                                                                                                                                                                                                                                         |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GC-MS:                                                                                                                                                                                                                | Instrument:         | HP6890/5973                                                                                                                                                                                                                                                                                                             |
|                                                                                                                                                                                                                       | Column:             | TG-1MS, 30 m x 0.25 mm I.D. x 0.25 µm                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                       | Program:            | 180 °C (1 min), 20 °C/min to 240 °C (10 min), 20 °C/min to 300 °C (2 min)                                                                                                                                                                                                                                               |
|                                                                                                                                                                                                                       | Injector:           | 250 °C                                                                                                                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                       | Split ratio:        | 30/1                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                                       | Transfer line temp: | 280 °C                                                                                                                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                       | Carrier:            | Helium, 1.0 mL/min                                                                                                                                                                                                                                                                                                      |
|                                                                                                                                                                                                                       | Scan range:         | 50-550 <i>m/z</i>                                                                                                                                                                                                                                                                                                       |
| The retention time of the parent compound 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.            |                     |                                                                                                                                                                                                                                                                                                                         |
| Parent (11.12 min): 290 ( <i>M</i> <sup>+</sup> , 84), 272 (100), 257 (100), 244 (78), 228 (32), 215 (63), 201 (56), 176 (17), 161 (56), 147 (56), 133 (41), 119 (51), 107 (77), 93 (81), 79 (96), 55 (69) <i>m/z</i> |                     |                                                                                                                                                                                                                                                                                                                         |
| The material was shown to co-elute with a previously registered sample of etiocholanolone (D551b) and also afford an identical mass spectrum.                                                                         |                     |                                                                                                                                                                                                                                                                                                                         |
| HS-GC-MS:                                                                                                                                                                                                             | Instrument:         | Agilent 6890/5973/G1888                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                                                                                       | Column:             | DB-624, 30 m x 0.25 mm I.D. x 1.4 µm                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                                       | Program:            | 50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)                                                                                                                                                                                                                                                        |
|                                                                                                                                                                                                                       | Injector:           | 150 °C                                                                                                                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                       | Transfer line temp: | 280 °C                                                                                                                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                       | Carrier:            | Helium, 1.2 mL/min                                                                                                                                                                                                                                                                                                      |
|                                                                                                                                                                                                                       | Split ratio:        | 50/1                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                                       | Solvents detected:  | Ethyl acetate                                                                                                                                                                                                                                                                                                           |
| TLC:                                                                                                                                                                                                                  | Conditions:         | Kieselgel 60F <sub>254</sub> . Chloroform/ethyl acetate (4/1)<br>Single spot observed, <i>R<sub>f</sub></i> = 0.2-0.3. Visualisation with vanillin                                                                                                                                                                      |
| IR:                                                                                                                                                                                                                   | Instrument:         | Biorad FTS300MX FT-IR                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                       | Range:              | 4000-400 cm <sup>-1</sup> , KBr powder                                                                                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                       | Peaks:              | 3469, 2937, 2866, 2850, 1729, 1718, 1456, 1364, 1045, 1009 cm <sup>-1</sup>                                                                                                                                                                                                                                             |
| <sup>1</sup> H NMR:                                                                                                                                                                                                   | Instrument:         | Bruker Avance 400                                                                                                                                                                                                                                                                                                       |
|                                                                                                                                                                                                                       | Field strength:     | 400 MHz                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                                                                                       | Solvent:            | CDCl <sub>3</sub> (7.26 ppm)                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                                                       | Spectral data:      | δ 0.84 (3H, s), 0.94 (3H, s), 1.00 (1H, ddd, <i>J</i> = 3.4, 14.3, 14.3 Hz), 1.13-1.95 (19H, m), 2.07 (1H, dt, <i>J</i> = 9.1, 19.3 Hz), 2.43 (1H, ddd, <i>J</i> = 0.9, 8.3, 19.3 Hz), 3.63 (1H, heptet, <i>J</i> = 4.8 Hz) ppm<br>Ethyl acetate estimated at 0.9% mass fraction was observed in the <sup>1</sup> H NMR |
| <sup>13</sup> C NMR:                                                                                                                                                                                                  | Instrument:         | Bruker Avance 400                                                                                                                                                                                                                                                                                                       |
|                                                                                                                                                                                                                       | Field strength:     | 100.6 MHz                                                                                                                                                                                                                                                                                                               |
|                                                                                                                                                                                                                       | Solvent:            | CDCl <sub>3</sub> (77.0 ppm)                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                                                       | Spectral data:      | δ 13.8, 20.1, 21.8, 23.3, 25.3, 26.9, 30.5, 31.7, 34.7, 35.3, 35.4, 35.9, 36.3, 40.7, 42.0, 47.8, 51.5, 71.6, 221.3 ppm                                                                                                                                                                                                 |
| Melting point:                                                                                                                                                                                                        |                     | 150-151 °C                                                                                                                                                                                                                                                                                                              |
| Microanalysis:                                                                                                                                                                                                        | Found:              | C = 78.7%; H = 10.4% (August, 2010)                                                                                                                                                                                                                                                                                     |
|                                                                                                                                                                                                                       | Calculated:         | C = 78.6%; H = 10.4% (Calculated for C <sub>19</sub> H <sub>30</sub> O <sub>2</sub> )                                                                                                                                                                                                                                   |