REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D708.2016.01 (Bottled 150210)

This batch of bottles was prepared from the bulk material on 10th February 2015.

Compound Name: 17α-Trenbolone  
Description: Light yellow powder

Collection Number: D708  
Batch Number: 01-AV-09

Chemical Formula: C₁₈H₂₂O₂  
Molecular Weight: 270.4

CAS Registry Number: 80657-17-6  
Release Date: 10th April 2002

Structure: Metabolite of trenbolone

Synonyms: Epitrenbolone, 17α-Hydroxyestra-4,9,11-trien-3-one

Purity (mass fraction): 98.5 ± 0.5% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR. Supporting evidence is provided elemental microanalysis.

**GC-FID:**
- **Instrument:** Agilent 6890N
- **Column:** HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
- **Program:** 180 °C (1 min), 15 °C/min to 250 °C (3 min), 30 °C/min to 310 °C (5 min)
- **Injector:** 250 °C
- **Carrier:** Helium
- **Detector Temp:** 320 °C
- **Relative peak area response of main component:**
  - **Initial analysis:** Mean = 99.2%, s = 0.08% (10 sub samples in duplicate, September 2001)
  - **Re-analysis:** Mean = 98.9%, s = 0.02% (5 samples in duplicate, June 2008)

**GC-FID:**
- **Instrument:** Agilent 6890N
- **Column:** HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
- **Program:** 180 °C (1 min), 10 °C/min to 250 °C (5 min), 30 °C/min to 310 °C (5 min)
- **Injector:** 250 °C
- **Carrier:** Helium
- **Detector Temp:** 320 °C
- **Relative peak area response of main component:**
  - **Initial analysis:** Mean = 99.0%, s = 0.1% (5 sub samples in duplicate, June 2011)
  - **Re-analysis:** Mean = 98.8%, s = 0.08% (5 sub samples in duplicate, March 2016)

**HPLC:**
- **Instrument:** Waters HPLC
- **Column:** Alltima C-18, 5 μm (4.6 mm × 150 mm)
- **Mobile Phase:** Methanol/water (60:40)
- **Flow Rate:** 1.0 mL/min
- **Detector:** UV at 340 nm
- **Initial analysis:** Mean = 99.8%, s = 0.01% (3 sub samples in duplicate, October 2001)

**Thermogravimetric analysis:**
- Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (November 2001 & June 2006)

**Karl Fischer analysis:**
- Moisture content 0.29% mass fraction (4 sub samples, May & June 2008)
- Moisture content 0.17% mass fraction (2 sub samples, June 2011)
- Moisture content 0.16% mass fraction (2 sub samples, April 2016)
**Spectroscopic and other characterisation data**

<table>
<thead>
<tr>
<th>Method</th>
<th>Details</th>
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<tbody>
<tr>
<td><strong>GC-MS</strong></td>
<td>Instrument: HP 5890/5972</td>
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<tr>
<td></td>
<td>Column: HP5-MS, 12 m x 0.25 mm I.D. x 0.11 μm</td>
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<td>Program: 150 °C (2.0 min), 10 °C /min to 280 °C</td>
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<td>Injector: 180 °C, Transfer line temp: 300 °C</td>
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<td>Carrier: Helium, 1 mL/min, Split ratio: N/A</td>
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<td>The retention time of the parent material is 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. 11.6 min: 270 (M⁺, 100), 252 (99), 237 (51), 155 (69), 141 (79), 128 (76), 115 (68) m/z</td>
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| **TLC**         | Conditions: Kieselgel 60F₂₅₄, Hexanes/Ethyl acetate (2:3) |
|                 | Single spot observed, Rₛ = 0.34 (3 samples) |

| **IR**          | Instrument: Perkin-Elmer FT-IR |
|                 | Range: 4000-400 cm⁻¹, KBr disc |
|                 | Peaks: 3425, 2942, 1639, 1569, 1278, 1233, 1087, 1029, 862, 765 cm⁻¹ |

| **¹H NMR**      | Instrument: Bruker DMX-500 |
|                 | Field strength: 500 MHz |
|                 | Solvent: CDCl₃ (7.26 ppm) |
| Key spectral data: δ 0.79 (3H, s), 1.35 (2H, m), 1.58 (1H, ddd), 2.43 (2H, t), 3.95 (1H, d), 5.75 (1H, s), 6.38 (1H, d), 6.54 (1H, d) ppm |

| **¹³C NMR**     | Instrument: Bruker DMX-600 |
|                 | Field strength: 126 MHz |
|                 | Solvent: CDCl₃ (77.2 ppm) |
| Spectral data: δ 18.5, 23.5, 24.2, 27.6, 31.5, 32.9, 36.6, 37.6, 46.1, 48.4, 78.0, 123.5, 125.4, 127.1, 141.3, 142.1, 156.7, 199.3 ppm |

| Melting point   | 106-109 °C |

| Microanalysis   | Found: C = 80.1%, H = 8.4% (July 2001) |
|                 | Calc: C = 80.0%, H = 8.2% (Calculated for C₁₈H₂₂O₂) |
Expiration of certification
The property values are valid till 31\textsuperscript{st} March, 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house 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 using gas chromatography with flame ionisation detection on ten 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.

STABILITY WARNING: Solutions prepared from this material are not stable when exposed to air or light. Where possible they should be used immediately. If this is not convenient, store solutions out of direct light at or below 4 \degree C and monitor regularly for possible decomposition.

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

Intended Use
For \textit{in vitro} laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI’s behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

\begin{center}
\large S. R. Davies
\end{center}

Dr Stephen R Davies
Team Leader,
Chemical Reference Materials, NMI
Dated: April 7, 2016

Characterisation data and property values specified in this report supersede those in all reports issued prior to 7\textsuperscript{th} April, 2016.