NMIA S016: 3’-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one

Report ID: S016.2019.01 (Ampouled 121025)

Chemical Formula: C_{20}H_{28}N_{2}O_{2}
Molecular Weight: 328.5 g/mol

Reference value

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>CAS No.</th>
<th>Mass per ampoule</th>
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<tbody>
<tr>
<td>12-S-04</td>
<td>1173998-80-5</td>
<td>781 μg</td>
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Expiration of certification: The property values are valid till 3 December 2022, i.e. three 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 S016. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile). This will transfer approximately 781 μg of 3’-hydroxy-5α-androstan[3,2-c]pyrazol-17-one.

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 been shown to decompose over time (see HPLC summary). 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.
This report supersedes any issued prior to 07 January 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 Alliance 2695 Separations Module
- Column: Alltima C-18, 5 μm (4.6 mm x 150 mm)
- Column oven: 40 °C
- Mobile Phase: Acetonitrile/MilliQ water (30:70 v/v) or
  A = MilliQ water; B = Acetonitrile 0-24 min 30% B; 24-25 min 30-70% B; 25-35 min
  70% B; 35-36 min 70-30% B; 36-45 min 30% B
- Flow rate: 1 mL/min
- Detector: Waters 2998 PDA operating at 250 nm

Relative peak area of the main component:
- Initial analysis: Mean = 99.7%, s = 0.03% (7 ampoules in duplicate, October 2012)
- Re-analysis: Mean = 99.4%, s = 0.2% (5 ampoules in duplicate, October 2013)
- Re-analysis: Mean = 99.1%, s = 0.2% (5 ampoules in duplicate, October 2014)
- Re-analysis: Mean = 97.4%, s = 1.4% (5 ampoules in duplicate, November 2015)
- Re-analysis: Mean = 95.9%, s = 0.4% (5 ampoules in duplicate, December 2016)
- Re-analysis: Mean = 94.5%, s = 0.3% (5 ampoules in duplicate, December 2019)

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 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.

\[
Purity = (100 \% - I_{ORG}) \times (100 \% - I_{VOL} - I_{NVR}) \quad \text{Equation 1}
\]

\[I_{ORG}\] = Organic impurities of related structure, \[I_{VOL}\] = volatile impurities, \[I_{NVR}\] = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

HPLC:
- Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
- Column: Alltima C-18, 5 μm (4.6 mm x 150 mm)
- Column oven: 40 °C
- Mobile Phase: Acetonitrile/MilliQ water (30:70 v/v)
- Flow rate: 1 mL/min
- Detector: Waters 2998 PDA operating at 250 nm

Relative peak area of the main component:
- Initial analysis: Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, July 2012)

Thermogravimetric analysis:
- Volatile content 11.2% and non volatile residue < 0.2% mass fraction (July 2012)

Karl Fischer analysis:
- Moisture content 17.6% mass fraction (July 2012)
Spectroscopic and other characterisation data

**LC-MS:**
- **Instrument:** Waters 2795 (HPLC)/Micromass Quatro
- **Column:** Prevail C-18, 50 mm × 2.1 mm I.D. × 3.5 μm
- **Column temp:** 32 °C
- **Solvent system:**
  - Solvent A: 10% Formic acid in Milli Q water
  - Solvent B: Milli Q water
  - Solvent C: Acetonitrile
- **Gradient elution:** 0 min 10% A 90% B; 1 min 10% A 90% B, 6.5 min 10% A 10% B 80% C; 8 min 10% A 90% B; 11 min 10% A 90% B
- **Flow rate:** 0.2 mL/min
- **Sample prep:** 50 μg/g in acetonitrile/MilliQ water
- **Injection volume:** 10 μL
- **Ionisation mode:** Electrospray positive ion
- **Capillary voltage:** 3.4 kV
- **Cone voltage:** 54 V
- **Source temp:** 120 °C
- **Desolvation gas temp:** 300 ºC
- **Cone gas flow rate:** 52 L/hr
- **Desolvation gas flow:** 550 L/hr

The retention time of 3'-hydroxy-5α-androstan[3,2-c]pyrazol-17-one is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.

8.68 min: 329.06 (M+H+) m/z

**TLC:**
- **Conditions:** Kieselgel 60F254. Ethyl acetate/methanol (95/5)
- Single spot observed, Rf = 0.15. Visualisation with UV at 254 nm

**IR:**
- **Instrument:** Biorad FTS3000MX FT-IR
- **Range:** 4000-400 cm⁻¹, KBr powder
- **Peaks:** 3525, 3233, 3079, 2935, 1711, 1599, 1453, 1380 cm⁻¹

**1H NMR:**
- **Instrument:** Bruker Avance-400
- **Field strength:** 400 MHz
- **Solvent:** DMSO-d6 (2.50 ppm)
- **Spectral data:** δ 0.68 (3H, s), 0.80 (3H, s), 0.85 (1H, m), 0.95 (1H, m), 1.15-1.89 (13H, m), 1.96-2.09 (2H, m), 2.28-2.42 (3H, m), 10.17 (2H, bs) ppm

**13C NMR:**
- **Instrument:** Bruker Avance-400
- **Field strength:** 101 MHz
- **Solvent:** DMSO-d6 (39.5 ppm)
- **Spectral data:** δ 11.5, 13.4, 20.1 21.5, 25.8, 28.4, 30.2, 31.4, 33.4, 34.7, 35.4, 36.0, 41.5, 47.0, 50.6, 53.3, 97.8, 138.5, 158.8, 219.9 ppm

**Microanalysis:**
- **Found:** C = 60.5%; H = 9.0%; N = 7.1% (July, 2012)
- **Calculated:** C = 73.1%; H = 8.6%; N = 8.5% (Calculated for C20H30N2O2)
- **Calculated:** C = 60.5%; H = 9.0%; N = 7.1% (Calculated for C20H30N2O2 + 17% water)