



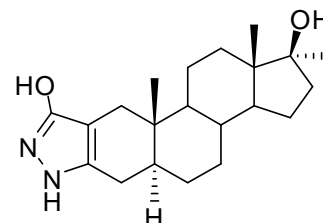
## REFERENCE MATERIAL PRODUCT INFORMATION SHEET

### NMIA D577: 3'-Hydroxystanozolol

Report ID: D577.2023.01 (Ampouled 120320)

Chemical Formula: C<sub>21</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>

Molecular Weight: 344.5 g/mol



#### Property value

| Batch No. | CAS No.     | Mass per ampoule |
|-----------|-------------|------------------|
| 99-S-06   | 125709-39-9 | 899 ± 43 µg      |

**IUPAC name:** (1S,3aS,3bR,5aS,10aS,10bS,12aS)-1,10a,12a-Trimethyl-1,2,3,3a,3b,4,5,5a,6,7,10,10a,10b,11,12,12a-hexadecahydrocyclopenta[5,6]naphtho[1,2-f]indazole-1,9-diol.

**Expiration of certification:** The property values are valid till 27 March 2028, 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 D577. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

**Intended use:** This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the assigned purity value to the SI unit for mass (kg) has not been established.

**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 approximately 899 ± 43 µg of anhydrous 3'-hydroxystanozolol.

**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.

**Stability:** This material has demonstrated stability over a period of six years. 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.  
13 April 2023

This report supersedes any issued prior to 13 April 2023.

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:

|       |   |  |
|-------|---|--|
| HPLC: | Instrument:                               | Waters Model 1525 Binary pump, 717 plus auto sampler or Thermo Scientific Ultimate 3000 RS Pump, RS auto sampler |
|       | Column:                                   | Alltima C-18, 5 µm (4.6 mm × 150 mm)   |
|       | Column oven:                              | 50 °C  |
|       | Mobile Phase:                             | Acetonitrile/20 mM ammonium acetate, pH 4.2 (37:63)  |
|       | Flow rate:                                | 1.0 mL/min   |
|       | Detector:                                 | Waters 2998 PDA or RS Diode Array Detector operating at 248 nm   |
|       | Relative peak area of the main component: |  |
|       | Initial analysis:                         | Mean = 98.3%, s = 0.3% (7 ampoules in duplicate, March 2012)   |
|       | Re-analysis:                              | Mean = 98.3%, s = 0.03% (4 ampoules in duplicate, February 2013)   |
|       | Re-analysis:                              | Mean = 98.9%, s = 0.07% (5 ampoules in duplicate, February 2014)   |
|       | Re-analysis:                              | Mean = 96.8%, s = 0.3% (6 ampoules in duplicate, February 2017)  |
|       | Re-analysis:                              | Mean = 97.2%, s = 0.2% (5 ampoules in duplicate, February 2018)  |
|       | Re-analysis:                              | Mean = 96.0%, s = 0.3% (5 ampoules in duplicate, November 2020)  |
|       | Re-analysis:                              | Mean = 95.6%, s = 0.16% (5 ampoules in duplicate, March 2023)  |

### 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 at 248 nm, thermogravimetric analysis, and Karl Fischer analysis. All organic impurities are assumed to have identical molar extinction coefficients at 248 nm and molecular weights as 3'-hydroxystanozolol. 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 elemental microanalysis.

|       |   |  |
|-------|---|--|
| HPLC: | Instrument:                               | Waters Model 1525 Binary pump, 717 plus auto sampler                 |
|       | Column:                                   | Alltima C-18, 5 µm (4.6 mm × 150 mm)                                 |
|       | Column oven:                              | 50 °C  |
|       | Mobile Phase:                             | Acetonitrile/20 mM ammonium acetate, pH 4.2 (37:63)                  |
|       | Flow rate:                                | 1.0 mL/min   |
|       | Detector:                                 | Waters 2998 PDA operating at 248 nm                                  |
|       | Relative peak area of the main component: |  |
|       | Initial analysis:                         | Mean = 99.9%, s = 0.02% (10 sub samples in duplicate, November 1999) |
|       | Re-analysis:                              | Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, April 2005)     |
|       | Re-analysis:                              | Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, March 2012)     |

Karl Fischer analysis: Moisture content 6.6% mass fraction (December 2007)  
Moisture content 6.0% mass fraction (March 2012)

Thermogravimetric analysis: Volatiles content 2.5 % and non-volatile residue < 0.2% mass fraction (December 2007)

## Spectroscopic and other characterisation data

|                      |  |   |
|----------------------|--|---|
| GC-MS:               | <i>Tris</i> -TMS derivative:   |   |
|                      | Instrument:  | Agilent 6890/5973   |
|                      | Column:  | HP Ultra 1, 17 m x 0.20 mm I.D. x 0.10 $\mu$ m  |
|                      | Program:   | 170 $^{\circ}$ C (0.5 min), 3 $^{\circ}$ C/min to 234 $^{\circ}$ C, 10 $^{\circ}$ C/min to 265 $^{\circ}$ C (3 min)                         |
|                      | Injector:  | 280 $^{\circ}$ C  |
|                      | Split ratio:   | 15/1  |
|                      | Transfer line temp:  | 300 $^{\circ}$ C  |
|                      | Carrier:   | Helium, 1.0 mL/min  |
|                      | Scan range:  | 50-550 <i>m/z</i>   |
|                      | The retention time of the <i>tris</i> -TMS derivative 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. |   |
|                      | <i>Tris</i> -TMS (16.7 min):   | 560 ( $M^+$ , 36), 545 (47), 254 (56), 143 (100), 73 (99)   |
| ESI-MS:              | Instrument:  | Finnigan MAT TSQ 700 with electrospray interface  |
|                      | Operation:   | Positive ion mode and negative ion mode, direct infusion at 5 $\mu$ L/min   |
|                      | Ionisation:  | ESI spray voltage at 3.5 kV positive ion mode, at 3.0 kV negative ion mode  |
|                      | EM voltage:  | 650 V   |
|                      | Cone voltage:  | 10 V  |
|                      | Peak:  | 403.2, 390.3, 373.3, 343.3 (M-H, 100) <i>m/z</i> (negative ion mode)<br>345.2 ( $MH^+$ , 100) <i>m/z</i> (positive ion mode)                |
| TLC:                 | Conditions:  | Kieselgel 60F <sub>254</sub> . Chloroform/methanol (90:10)<br>Single spot observed, $R_f$ = 0.1-0.2. Visualisation with UV at 254 nm        |
| IR:                  | Instrument:  | FT-IR, Biorad WIN FTS40   |
|                      | Range:   | 4000-400 $cm^{-1}$ , KBr pellett  |
|                      | Peaks:   | 3360, 1617, 1445, 1296, 1149, 1102, 937 $cm^{-1}$   |
| <sup>1</sup> H NMR:  | Instrument:  | Bruker ARX-500  |
|                      | Field strength:  | 500 MHz   |
|                      | Solvent:   | DMSO- <i>d</i> <sub>6</sub> (2.50 ppm)  |
|                      | Key spectral data:   | $\delta$ 0.66 (3H, s), 0.74 (3H, s), 1.07 (3H, s) ppm   |
| <sup>13</sup> C NMR: | Instrument:  | Bruker Avance III-500   |
|                      | Field strength:  | 126 MHz   |
|                      | Solvent:   | DMSO- <i>d</i> <sub>6</sub> (39.52 ppm)   |
|                      | Spectral data:   | $\delta$ 11.5, 14.1, 20.4, 23.2, 25.8, 26.1, 28.6, 31.2, 31.4, 33.4, 35.9, 36.2, 38.3, 41.6, 45.0, 50.1, 53.3, 79.8, 97.9, 138.5, 158.8 ppm |
| Melting point:       | 298-306 $^{\circ}$ C   |   |
| Microanalysis:       | Found:   | C = 73.0%; H = 9.2%; N = 8.2 % (April 1999)   |
|                      | Calculated:  | C = 73.2%; H = 9.4%; N = 8.1% (Calculated for C <sub>21</sub> H <sub>32</sub> N <sub>2</sub> O <sub>2</sub> )                               |