



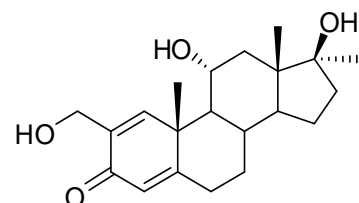
## REFERENCE MATERIAL PRODUCT INFORMATION SHEET

### NMIA D622: 2-Hydroxymethyl-17 $\alpha$ -methylandrosta-1, 4-diene-11 $\alpha$ , 17 $\beta$ -diol-3-one

Report ID: D622.2024.01 (Ampouled 191031)

Chemical Formula: C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>

Molecular Weight: 346.5 g/mol



#### Property value

Batch No.	CAS No.	Mass per ampoule
99-S-29	59400-02-1	1029 ± 71 µg

**IUPAC name:** (11 $\alpha$ ,17 $\beta$ )-11,17-Dihydroxy-2-(hydroxymethyl)-17-methylandrosta-1,4-dien-3-one.

**Expiration of certification:** The property values are valid until 17 April 2029, five 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.

**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 D622. 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 1029 ± 71 µg of anhydrous 2-hydroxymethyl-17 $\alpha$ -methylandrosta-1,4-diene-11 $\alpha$ ,17 $\beta$ -diol-3-one.

**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:** 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.  
1 May 2024

This report supersedes any issued prior to 01 May 2024.

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: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm  $\times$  150 mm)  
 Column oven: 40  $^{\circ}$ C  
 Mobile Phase: A = MilliQ water; B = Acetonitrile  
 0-12 min 26% B; 12-13 min 26-50% B; 13-18 min 50%B; 18-19 min 50-26%B,  
 19-25 min 26% B  
 Flow rate: 1.0 mL/min  
 Detector: Shimadzu SPD-M20A PDA operating at 254 nm  
 Relative peak area of the main component:  
 Initial analysis: Mean = 94.8%, s = 0.04% (7 ampoules in duplicate, November 2019)  
 Re-analysis: Mean = 94.8%, s = 0.03% (5 ampoules in duplicate, April 2024)

**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  $^1$ 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 elemental microanalysis.

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm  $\times$  150 mm)  
 Mobile Phase: Acetonitrile/water (26:74)  
 Flow Rate: 1.0 mL/min  
 Detector: Waters PDA 996 operating at 254 nm  
 Relative peak area of the main component:  
 Initial analysis: Mean = 96.0%, s = 0.2% (10 sub samples in duplicate, February 2000)  
 Re-analysis: Mean = 96.0%, s = 0.04% (10 sub samples, June 2009)

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm  $\times$  150 mm)  
 Column oven: 40  $^{\circ}$ C  
 Mobile Phase: A = MilliQ water; B = Acetonitrile  
 0-12 min 26% B; 12-13 min 26-50% B; 13-18 min 50% B; 18-19 min 50-26%B,  
 19-25 min 26% B  
 Flow rate: 1.0 mL/min  
 Detector: Shimadzu SPD-M20A PDA operating at 254 nm  
 Relative peak area of the main component:  
 Initial analysis: Mean = 94.9%, s = 0.02% (7 sub samples in duplicate, November 2019)

Karl Fischer analysis: Moisture content 5.2% mass fraction (May 2009)  
 Moisture content 5.1% mass fraction (November 2019)

Thermogravimetric analysis: Volatiles content 4.8% (February 2000 & November 2005)  
 Non-volatile residue 0.3% mass fraction (June 2009)

**Spectroscopic and other characterisation data**

GC-MS: Parent compound:  
 Instrument: HP6890/5973  
 Column: HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11  $\mu$ m  
 Program: 140  $^{\circ}$ C (1 min), 8  $^{\circ}$ C/min to 250  $^{\circ}$ C, 30  $^{\circ}$ C/min to 300  $^{\circ}$ C (3 min)  
 Injector: 280  $^{\circ}$ C  
 Split ratio: Splitless  
 Transfer line temp: 300  $^{\circ}$ C  
 Carrier: Helium  
 Scan range: 50-550 *m/z*

*Tris*-TMS derivative:

Instrument: HP6890/5973  
 Column: HP Ultra 1, 17 m x 0.22 mm ID x 0.11  $\mu$ m  
 Program: 170  $^{\circ}$ C, 3  $^{\circ}$ C/min to 234  $^{\circ}$ C, 10  $^{\circ}$ C/min to 265  $^{\circ}$ C (3 min)  
 Injector: 280  $^{\circ}$ C  
 Transfer line temp: 300  $^{\circ}$ C  
 Carrier: Helium  
 Split ratio: 15/1  
 Scan range: 50-550 *m/z*

The retention times of the parent compound and *Tri*-TMS derivative are 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 (8.6 min): 328 ( $M^+$ -H<sub>2</sub>O, 100), 310 (30), 295 (16), 281 (14), 257 (22), 239 (25), 91 (50) *m/z*

*Tris*-TMS (16.5 min): 562 ( $M^+$ , 3), 547 (2), 457 (6), 367 (12), 207 (17), 143 (29), 73 (100) *m/z*

TLC: Conditions: Kieselgel 60F<sub>254</sub>. Methanol /ethyl acetate (1:10)  
 Single spot observed, R<sub>f</sub> = 0.4 (5 sub samples)

IR: Instrument: Perkin-Elmer FT-IR  
 Range: 4000-400  $\text{cm}^{-1}$ , Nujol mull  
 Peaks: 3448, 3284, 1655, 1614, 1449, 1373, 1149, 1032, 908, 732  $\text{cm}^{-1}$

<sup>1</sup>H NMR: Instrument: Bruker Avance - 300  
 Field strength: 300 MHz  
 Solvent: MeOH-d<sub>4</sub> (3.31 ppm)  
 Spectral data:  $\delta$  0.95 (3H, s), 1.18 (3H, s), 1.36 (3H, s), 4.10 (1H, m), 4.28 (2H, m), 6.14 (1H, s), 8.05 (1H, s) ppm

<sup>13</sup>C NMR: Instrument: Bruker Avance - 300  
 Field strength: 75 MHz  
 Solvent: MeOH-d<sub>4</sub> (49.0 ppm)  
 Spectral data:  $\delta$  16.0, 19.6, 24.7, 26.5, 34.3, 35.2, 37.2, 39.7, 44.4, 46.1, 47.6, 51.0, 60.8, 62.5, 69.3, 81.9, 125.0, 134.7, 158.2, 172.7, 188.8 ppm

Melting point: 153-154.5  $^{\circ}$ C

Microanalysis: Found: C = 69.4%; H = 9.1% (November, 1999)  
 Calculated: C = 69.2%; H = 8.9% (Calculated for C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>·H<sub>2</sub>O)