



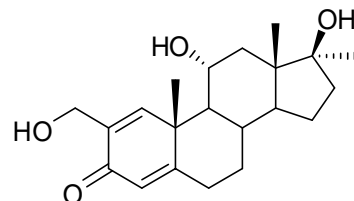
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D622: 2-Hydroxymethyl-17 α -methylandrosta-1, 4-diene-11 α , 17 β -diol-3-one

Report ID: D622.2019.01 (Ampouled 191031)

Chemical Formula: C₂₁H₃₀O₄

Molecular Weight: 346.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-29	59400-02-1	1039 μ g

IUPAC name: (11 α ,17 β)-11,17-Dihydroxy-2-(hydroxymethyl)-17-methylandrosta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 8 November 2024, i.e. 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 1039 μ g of anhydrous 2-hydroxymethyl-17 α -methylandrosta-1,4-diene-11 α ,17 β -diol-3-one.

Recommended storage: When not in use, this material should be stored at or below 4 $^{\circ}$ 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.
6 December 2019

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: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
 Column: Alltima C-18, 5 μ 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 = 95.8%, s = 0.04% (7 ampoules in duplicate, November 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 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_{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 μ 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 μ 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 = 95.9%, 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 μ 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 <i>m/z</i>
	<i>Tris</i> -TMS derivative:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm ID x 0.11 μ 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 <i>m/z</i>
	The retention times of the parent compound and <i>Tri</i> -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 ₂ O, 100), 310 (30), 295 (16), 281 (14), 257 (22), 239 (25), 91 (50) <i>m/z</i>
	<i>Tris</i> -TMS (16.5 min):	562 (M^+ , 3), 547 (2), 457 (6), 367 (12), 207 (17), 143 (29), 73 (100) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Methanol /ethyl acetate (1:10) Single spot observed, R _f = 0.40 (5 sub samples)
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1} , Nujol mull
	Peaks:	3448, 3284, 1655, 1614, 1449, 1373, 1149, 1032, 908, 732 cm^{-1}
¹ H NMR:	Instrument:	Bruker Avance - 300
	Field strength:	300 MHz
	Solvent:	CD ₃ OD (3.31 ppm)
	Spectral data:	δ 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
¹³ C NMR:	Instrument:	Bruker Avance - 300
	Field strength:	75 MHz
	Solvent:	CD ₃ OD (49.0 ppm)
	Spectral data:	δ 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 ₂₁ H ₃₀ O ₄ .H ₂ O)