



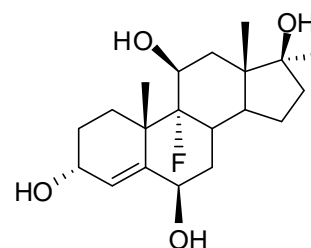
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D616: 9 α -Fluoro-17 α -methyl-4-androsten-3 α , 6 β , 11 β , 17 β -tetrol

Report ID: D616.2020.01 (Ampouled 080804)

Chemical Formula: C₂₀H₃₁FO₄

Molecular Weight: 354.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-24	148505-57-1	990 μ g

IUPAC name: (3 α ,6 β ,11 β ,17 β)-9-Fluoro-17-methylandro-4-ene-3,6,11,17-tetrol

Expiration of certification: The property values are valid till 16 June 2030, i.e. ten 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 under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D616. The 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.

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 990 μ g of anhydrous 9 α -fluoro-17 α -methyl-4-androsten-3 α , 6 β , 11 β , 17 β -tetrol. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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: This material has demonstrated stability over a minimum period of five 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/ELS 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.
23 June 2020

This report supersedes any issued prior to 23 June 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 Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μ m (4.6 mm \times 150 mm)
	Mobile Phase:	Water/Acetonitrile (A/B), Gradient: 0-6 min, 17% B, 6-8 min 17-40% B, 8-15 min 40% B, 15-16 min 40-17% B, 16-26 min 17% B
	Flow Rate:	0.8 mL/min
	Detector:	Waters PDA 2998 at 201 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 98.8%, s = 0.03% (7 ampoules in duplicate, August 2008)
	Re-analysis:	Mean = 98.7%, s = 0.03% (5 ampoules in duplicate, August 2009)
	Re-analysis:	Mean = 99.0%, s = 0.03% (5 ampoules in duplicate, May 2012)
	Re-analysis:	Mean = 99.1%, s = 0.06% (5 ampoules in duplicate, May 2015)
	Detector:	Waters ELSD 2420 or 2424
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (7 ampoules in duplicate, August 2008)
	Re-analysis:	Mean = 99.6%, s = 0.03% (5 ampoules in duplicate, August 2009)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, May 2012)
HPLC:	Instrument:	Waters Alliance 2695 Separations module
	Column:	Alltima C-18, 5 μ m (4.6 mm \times 150 mm)
	Mobile Phase:	Milli Q Water/Acetonitrile (A/B), Gradient: 0-6 min 17% B, 6-8 min 17-40% B, 8-15 min 40% B, 15-16 min 40-17% B, 16-26 min 17% B
	Flow Rate:	0.8 mL/min
	Detector:	Waters PDA 2998 at 201 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.0%, s = 0.01% (5 ampoules in duplicate, June 2020)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

Characterisation Report:

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 and evaporative light scattering detection, thermogravimetric analysis, Karl Fischer analysis and ^1H 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:	Column:	Alltima C-18, 5 μ m (4.6 mm \times 150 mm)
	Mobile Phase:	Water/Acetonitrile (A/B), Gradient: 0-6 min, 17% B, 6-8 min 17-40% B, 8-15 min 40% B, 15-16 min 40-17% B, 16-26 min 17% B
	Flow Rate:	0.8 mL/min
	Detector:	ELSD
	Relative peak area of main component:	
	Initial analysis:	Mean > 99.9% (3 sub samples, June 2000)
	Re-analysis:	Mean = 99.7% (5 sub samples in duplicate, August 2008)
	Detector:	UV at 201 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 98.4%, s = 0.02% (2 sub samples, October 1999)
	Current re-analysis:	Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, August 2008)
Karl Fischer analysis:		Moisture content 0.24% mass fraction (August 2008)
Thermogravimetric analysis:		Volatiles content 0.3% and non-volatile residue < 0.2% mass fraction (November 1999 & October 2005)

Spectroscopic and other characterisation data

GC-MS:	<i>Tetra</i> -TMS derivative: Instrument: HP 6890/5973 Column: HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11 μ 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: 20/1 Transfer line temp: 300 $^{\circ}$ C Carrier: Helium Scan range: 50-550 <i>m/z</i> The retention time of <i>tetra</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>Tetra</i> -TMS (13.5 min): 642 (M^+ , 2), 552 (7), 462 (4), 357 (3), 143 (86), 73 (80) <i>m/z</i>
TLC:	Conditions: Kieselgel 60F ₂₅₄ . Ethyl acetate Single spot observed, R_f = 0.3
IR:	Instrument: Perkin-Elmer FT-IR Range: 4000-400 cm^{-1} , Nujol mull Peaks: 3592, 3328, 2357, 1454, 1377, 1266, 994, 934 cm^{-1}
¹ H NMR:	Instrument: Bruker Avance III-500 Field strength: 500 MHz Solvent: DMSO- <i>d</i> ₆ (2.50 ppm) Spectral data: δ 1.00 (3H, s), 1.05, 1.42 (3H, s), 1.51 (3H, s), 3.89 (1H, br s), 3.99 (1H, br s), 4.1 (1H, br d), 5.50 (1H, d) ppm Signals consistent with the presence of the 3 β -hydroxy isomer are observed in the NMR spectrum. The amount of impurity was estimated at 1% mass fraction of D616.
¹³ C NMR:	Instrument: Bruker Avance III-500 Field strength: 126 MHz Solvent: DMSO- <i>d</i> ₆ (39.52 ppm) Spectral data: δ 16.3, 23.4, 25.2, 25.4, 26.7, 27.9, 31.2 (d), 34.4, 37.0, 38.5, 42.1 (d), 44.3, 45.2, 62.2, 69.7 (d), 71.8, 80.4, 101.0 (d), 127.5, 146.5 ppm
Melting point:	201-203 $^{\circ}$ C
Microanalysis:	Found: C = 67.7%, H = 8.8% Calculated: C = 67.8%, H = 8.8% (Calculated for C ₂₀ H ₃₁ FO ₄)