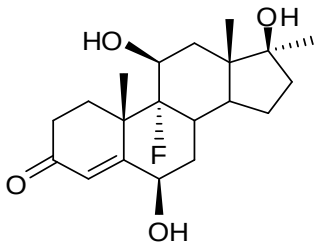
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D617: 6β-Hydroxyfluoxymesterone



Report ID: D617.2016.02 (Ampouled 100218)

Chemical Formula: $C_{20}H_{29}FO_4$

Molecular Weight: 352.5 g/mol

Property value

Batch No.	CAS No.	Mass per ampoule
99-S-22	**88936-08-7**	**994 μg**

IUPAC name: (6β,11β,17β)-9-Fluoro-6,11,17-trihydroxy-17-methylandrost-4-en-3-one

Expiration of certification: The property values are valid till 2 December 2021, 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 under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D617. 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 994 μg of anhydrous 6β-hydroxyfluoxymesterone. 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 ºC 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 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.
21 February 2020

This report supersedes any issued prior to 21 February 2020

Characterisation Report:

Warning: This material was ampouled by dissolution in methanol and subsequently dispensed into individual ampoules. Please note that heat (45 °C water bath) was applied to aid the dissolution of the material at 2 mg/mL.

HPLC:
Instrument: Waters HPLC 1525 pump, 717 autosampler
Column: Alltima C18, 5 µm (4.6 mm × 150 mm)
Mobile Phase: A: Water, B: Acetonitrile
Gradient: 25% B for 8 min, increase B to 50% in 7 min, elute for 4 min, then reduce B to 25% in 1 min, elute for another 10 min.
Flow Rate: 0.8 mL/min

Detector: ELSD
Relative peak area response of main component:
Initial analysis: Mean = 99.9%, s = 0.008% (7 ampoules in duplicate, April 2010)
Re-analysis: Mean = 99.6%, s = 0.1% (5 ampoules in duplicate, March 2011)

Detector: UV at 236.5 nm
Relative peak area response of main component:
Initial analysis: Mean = 98.8%, s = 0.02% (7 ampoules in duplicate, April 2010)
Re-analysis: Mean = 98.9%, s = 0.05% (5 ampoules in duplicate, March 2011)
Re-analysis: Mean = 98.9%, s = 0.07% (5 ampoules in duplicate, January 2014)
Re-analysis: Mean = 98.8%, s = 0.01% (5 ampoules in duplicate, December 2016)

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

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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 HPLC 1525 pump, 717 autosampler
Column: Alltima C18, 5 µm (4.6 mm × 150 mm)
Mobile Phase: Acetonitrile/ water (25:75)
Flow Rate: 0.8 mL/min

Detector: ELSD
Relative peak area response of main component:
Initial analysis: Mean = 100.0%, s = 0% (3 sub samples in duplicate, January 2000)
Re-analysis: Mean = 99.96%, s = 0.004% (5 sub samples in duplicate, May 2006)

Detector: UV at 236.5 nm
Relative peak area response of main component:
Initial analysis: Mean = 99.6%, s = 0.02% (3 sub samples in duplicate, June 2003)
Re-analysis: Mean = 99.6%, s = 0.007% (5 sub samples in duplicate, May 2006)

HPLC:
Instrument: Waters HPLC 1525 pump, 717 autosampler
Column: Alltima C18, 5 µm (4.6 mm × 150 mm)
Mobile Phase: A: Water, B: Acetonitrile
Gradient: 25% B for 8 min, increase B to 50% in 7 min, elute for 4 min, then reduce B to 25% in 1 min, elute for another 10 min
Flow Rate: 0.8 mL/min Retention time: 8.5 min

Detector: ELSD
Relative peak area response of main component:
Initial analysis: Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, March 2010)

Detector: UV at 236.5 nm
Relative peak area response of main component:
Initial analysis: Mean = 98.8%, s = 0.03% (7 sub samples in duplicate, March 2010)

Karl Fischer analysis: Moisture content < 0.2% mass fraction (February 2010)

Thermogravimetric analysis: Volatiles content < 0.1 and non-volatile residue < 0.2% mass fraction (January 2000, June 2005 and May 2006)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP6890/5973
	Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μm
	Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*

The retention time of the *tetra*-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.

Tetra-TMS (15.8 min): 640 (M^+, 100), 625 (2), 605 (1), 535 (2), 496 (2), 143, 73 (58) *m/z*

Formation of the *tetra*-TMS derivative is difficult, requiring prolonged heating at 70 °C with MSTFA. The tris-TMS derivative is observed as a separate peak in the chromatogram.

TLC:	Conditions:	Kieselgel $60F_{254}$. Ethyl acetate (5:3) Single spot observed, R_f = 0.4
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, Nujol mull
	Peaks:	3431, 3363, 1666, 1615, 1453, 1368, 1231, 1053, 942 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Advance-300
	Field strength:	300 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Key Spectral data:	δ 0.86 (3H, s), 0.89 (3H, s), 1.47 (3H, s), 4.69 (1H, br s), 4.95 (1H, br s), 5.54 (1H, br s) ppm
	^{13}C NMR: Instrument:	Bruker Advance-300
	Field strength:	75 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 16.2, 23.2, 23.3, 26.7, 29.2, 31.2 (d), 33.5, 33.8, 36.8, 38.5, 43.4 (d), 44.2, 45.0, 69.4 (d), 70.0, 80.3, 101.6 (d), 126.5, 168.5, 199.1 ppm
Melting point:		289 °C
Microanalysis:	Found:	C = 68.3%; H = 8.3% (August 1999)
	Calculated:	C = 68.2%; H = 8.3% (Calculated for $C_{20}H_{29}FO_4$)