Australian Government
Department of Industry, Science and Resources

National Measurement Institute

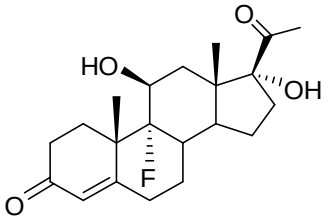
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D652: Fluorogestone

Report ID: D652.2020.03

Chemical Formula: $C_{21}H_{29}FO_4$

Molecular Weight: 364.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
00-AV-02	337-03-1	96.9 ± 2.2%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (11β)-9-Fluoro-11,17-dihydroxypregn-4-ene-3,20-dione

Expiration of certification: The property values are valid till 23 September 2025, 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 25 ºC in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 ten randomly selected 1-2 mg sub samples 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 a 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.
10 November 2022

This report supersedes any issued prior to 10 November 2022.

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.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The one-proton singlet at 5.6 ppm was measured against a certified internal standard of dimethyl terephalate.

Supporting evidence is provided by a range of traditional analytical techniques including HPLC with PDA and ELS detection, thermogravimetric analysis, Karl Fischer analysis and elemental microanalysis.

HPLC:	Instrument:	Waters Alliance 2695 Separations module
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-9 min 33% B, 9-11 min 33-90% B, 11-16 min 90% B, 16-17 min 90-33% B, 17-25 min 33% B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.02% (5 sub samples in duplicate, September 2020)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-9 min 35% B, 9-11 min 35-90% B, 11-16 min 90% B, 16-17 min 90-35% B, 17-25 min 35% B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.04% (5 sub samples in duplicate, January 2015)
	Re-analysis:	Mean = 98.7%, s = 0.01% (5 sub samples in duplicate, November 2017)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Waters Nova Pak C-18, 5 μm (3.9 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-9 min 35% B, 9-11 min 35-90% B, 11-16 min 90% B, 16-17 min 90-35% B, 17-25 min 35% B
	Flow rate:	1.0 mL/min
	Detector:	Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.55% (10 sub samples in duplicate, August 2000)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 sub samples in duplicate, October 2005)
HPLC:	Instrument:	Shimadzu binary pump LC-20AB, Shimadzu autosampler SIL-20A HT
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	45 °C
	Mobile Phase:	Methanol/MilliQ water (55:45 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.5%, s = 0.03% (5 sub samples in duplicate, December 2010)
	Detector:	Shimadzu SPD-M20A PDA operating at 240 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 98.6%, s = 0.01% (5 sub samples in duplicate, December 2010)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (December 2010, January 2015 and July 2020) Moisture content < 0.2 % mass fraction (November 2017)
Thermogravimetric analysis:		Volatile and non volatile residue < 0.2 % mass fraction (December 2010)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethyl terephthalate (100.0% mass fraction)
	Initial analysis:	Mean (5.6 ppm) = 96.9%, s = 0.43% (3 sub samples, February 2011)

Spectroscopic and other characterisation data

GC-MS:	*Tetra*-TMS compound:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 1, 17 m × 0.20 mm I.D. × 0.11 μm
	Program:	170 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	260 °C
	Split ratio:	40/1
	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 (10.9 min):	652 (M^+, 28), 422 (31), 231 (8), 147 (16), 73 (100) *m/z*
ESI-MS:	Instrument:	Finnigan TSQ-700
	Operation:	Positive ion mode
	Ionisation:	ESI spray voltage at 4.5 kV positive ion
	Peak:	365 ($M+H^+$) *m/z*
	Operation:	Negative ion mode
	Ionisation:	ESI spray voltage at 3.0 kV positive ion
	EM voltage:	650 V
	Peak:	423 $[M+CH3COO]^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (3:1) Single spot observed, R_f = 0.14
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, KBr disc
	Peaks:	3445, 3359, 1688, 1654, 1354, 1042 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.91 (3H, s), 1.62 (3H, s), 2.70 (3H, s), 4.30 (1H,dt, *J* = 3.5, 8.4 Hz), 5.79 (1H, d, *J* = 1.6 Hz) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 17.2, 22.4 (d), 24.3, 27.1, 27.4 (d), 29.5(d), 32.1, 33.5, 34.6, 35.5 (d), 37.3, 45.3 (d), 46.4, 46.9, 71.1 (d), 91.1, 101.2 (d), 124.9, 173.5, 202.0, 212.7 ppm
Melting point:		260 °C (decomposition)
Microanalysis:	Found:	C = 69.3%; H = 8.0% (October, 2000)
	Calculated:	C = 69.2%; H = 8.0% (Calculated for $C_{21}H_{29}FO_4$)