Australian Government
Department of Industry, Science and Resources

National Measurement Institute

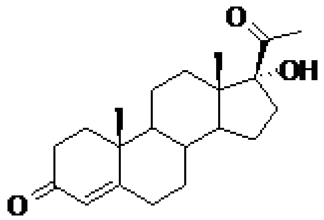
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S041: 17α-Hydroxyprogesterone

Report ID: S041.2021.03 (Bottled 201021)

Chemical Formula: $C_{21}H_{30}O_3$

Molecular Weight: 330.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
16-S-04	**68-96-2**	**98.7 ± 0.8%**

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

IUPAC name: 17-Hydroxypregn-4-ene-3,20-dione.

Expiration of certification: The property values are valid till 8 March 2026, 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: Off-white solid sourced from an external supplier, 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 4 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. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. 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 3 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.
1 November 2022

This report supersedes any issued prior to 1 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see http://www.bipm.org/kcdb/). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

The certified purity value by qNMR was obtained using the three-proton singlet at 0.71 ppm and the one-proton singlet at 5.87 ppm measured against a certified internal standard of dimethyl fumarate.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Thermo Scientific Ultimate 3000 RS Pump or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40)
	Flow rate:	1.0 mL/min
	Detector:	RS Diode Array or Shimadzu SPD-M20A PDA Detector operating at 246 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.1%, s = 0.03% (10 sub samples in duplicate, April 2016)
	Re-analysis:	Mean = 99.2%, s = 0.11% (5 sub samples in duplicate, May 2018)
	Re-analysis:	Mean = 99.1%, s = 0.03% (5 sub samples in duplicate, March 2021)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (April 2016) Moisture content 0.1% mass fraction (January 2017) Moisture content < 0.1% mass fraction (March 2018 and 2021)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (June 2016)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	AcOH-d_4 (2.50 ppm)
	Internal standard:	Dimethyl fumarate (99.9% mass fraction)
	Initial analysis:	Mean (0.71 ppm) = 98.5%, s = 0.1% (5 sub samples, April 2017)
	Initial analysis:	Mean (5.87 ppm) = 98.3%, s = 0.1% (5 sub samples, April 2017)

Spectroscopic and other characterisation data

GC-MS: *Tris*-TMS derivative:
Instrument: Agilent 6890/5973
Column: HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program: 180 °C (0.5 min), 12 °C/min to 300 °C (3 min)
Injector: 260 °C Split ratio: 20/1
Transfer line temp: 280 °C
Carrier: Helium, 1.0 mL/min
Scan range: 50-550 *m/z*

The retention time of the *tris*-TMS derivative is reported with the major peak in the mass spectra. The latter is reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Tris-TMS (15.8 min): 546 (M$^+$, 95), 456 (17), 441 (17), 316 (51), 301 (39), 231 (20), 208 (17), 193 (12), 155 (11), 147 (30), 73 (100) *m/z*

LC-MS: Instrument: Water ACQUITY UPLC/TQ Detector
Column: Porshell 120 EC-C18, 100 mm × 2.1 mm I.D. × 2.7 μm
Column temp: 40 °C
Solvent system: MilliQ water with 0.1% formic acid [50% v/v], Methanol [50% v/v]
Flow rate: 0.2 mL/min
Sample prep: 50 μg/g in MeOH
Injection volume: 3 μL
Ionisation mode: Electrospray positive ion
Capillary voltage: 3.5 kV Cone voltage: 20 V
Source temp: 120 ºC Desolvation gas temperature: 350 ºC
Cone gas flow rate: 1 L/hr Desolvation gas flow rate: 400 L/hr

The retention time of 17α-hydroxyprogesterone is reported with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.

Parent (6.98 min): 331.2 (M+H$^+$) *m/z*

HS-GC-MS: Instrument: Agilent 6890/5973/G1888
Column: DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
Program: 50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
Injector: 150 °C
Transfer line temp: 280 °C
Carrier: Helium, 1.2 mL/min
Split ratio: 50/1
Solvents detected: No solvents detected.

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1)
Single spot observed, R_f = 0.33. Visualisation with UV at 254 nm

IR: Instrument: Bruker Alpha Platinum ATR
Range: 4000-400 cm^{-1}, neat
Peaks: 3420, 2931, 2915, 2859, 1701, 1662, 1612, 1352, 1230, 1191, 1122, 1095, 882, 589 cm^{-1}

^{1}H NMR: Instrument: Bruker Avance III -500
Field strength: 500 MHz
Solvent: $CDCl_3$ (7.26 ppm)
Spectral data: δ 0.75 (3H, s), 0.97 (1H, m), 1.10 (1H, m), 1.18 (3H, s), 1.30-1.45 (3H, m), 1.55-1.77 (6H, m), 1.79-1.89 (2H, m), 2.02 (1H, m), 2.22-2.46 (4H, m), 2.27 (3H, s), 2.68 (1H, m), 5.72 (1H, s) ppm

Ethanol estimated at < 0.1% mass fraction was observed in the ^{1}H NMR

^{13}C NMR: Instrument: Bruker Avance III -500
Field strength: 126 MHz
Solvent: $CDCl_3$ (77.19 ppm)
Spectral data: δ 15.5, 17.5, 20.6, 24.1, 28.0, 30.2, 32.1, 33.0, 33.6, 34.1, 35.6, 35.8, 38.7, 48.3, 50.1, 53.4, 89.9, 124.1, 171.3, 199.8, 211.8 ppm

Melting point: 213-218 °C

Microanalysis: Found: C = 76.3%; H = 9.3% (April 2016)
Calculated: C = 76.3%; H = 9.2% (Calculated for $C_{21}H_{30}O_3$)