Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

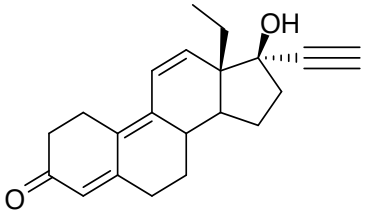
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D860b: Gestrinone

Report ID: D860b.2020.01

Chemical Formula: $C_{21}H_{24}O_2$

Molecular Weight: 308.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
08-S-13	**16320-04-0**	**97.7 ± 0.3%**

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

IUPAC name: (8S,13S,14S,17R)-13-Ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,13,14,15,16,17-decahydro-3H-cyclopenta[a]phenanthren-3-one.

Expiration of certification: The property values are valid till 12 May 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: Yellow powder prepared by 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%.

Stability: This material has demonstrated stability over a minimum period of three 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 GC-FID 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.
14 May 2020

This report supersedes any issued prior to 14 May 2020.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) 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.

Note: This material has shown signs of degradation when injected onto a silanised glass liner at elevated temperatures.

GC-FID:	Instrument:	Varian CP3800 or Agilent 6890N
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	150 °C (1 min), 30 °C/min to 240 °C (8 min), 30 °C /min to 300 °C (5 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 97.0%, s = 0.06% (10 sub samples in duplicate, July 2008)
	Re-analysis:	Mean = 98.0%, s = 0.07% (5 sub samples in duplicate, June 2014)
	Re-analysis:	Mean = 97.9%, s = 0.05% (5 sub samples in duplicate, May 2017)
	Re-analysis:	Mean = 97.8%, s = 0.04% (5 sub samples in duplicate, May 2020)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 240 °C (11 min), 40 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 97.9%, s = 0.01% (5 sub samples in duplicate, August 2009)
	Re-analysis:	Mean = 97.9%, s = 0.02% (5 sub samples in duplicate, July 2011)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (0.5 min), 8 °C/min to 235 °C (13 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 97.9%, s = 0.05% (5 sub samples in duplicate, July 2010)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (October 2008) Moisture content < 0.3% mass fraction (July 2009) Moisture content 0.3% mass fraction (July 2010 and July 2011) Moisture content 0.14% mass fraction (May 2014) Moisture content 0.2% mass fraction (May 2017) Moisture content 0.2% mass fraction (May 2020)
Thermogravimetric analysis:		Initial volatile content < 0.1% and non volatile residue < 0.2% mass fraction (October 2008 & August 2009)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	AT-5MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (1 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	30/1
	The retention time of the parent compound 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.	
	Parent (11.1 min):	308 (M^+, 83), 290 (12), 279 (62), 261 (100), 251 (18), 240 (30), 227 (87), 211 (39), 197 (33), 181 (35), 165 (45), 153 (42), 141 (52), 128 (42), 115 (39), 91 (29), 77 (19), 53 (16) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1) Single spot observed, R_f = 0.6. Visualisation with UV at 254 nm.
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3420, 3299, 3254, 2937, 2876, 2099, 1637, 1570, 1232, 1068, 880 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz
	Solvent:	MeOH-d_4 (3.30 ppm)
	Spectral data:	δ1.00 (3H, t, J = 7.4 Hz), 1.26 (1H, m), 1.44-1.54 (2H, m), 1.67-1.74 (2H, m), 1.91 (1H, m), 2.05-2.11 (2H, m), 2.30 (1H, m), 2.44 (2H, t, *J* = 7.5 Hz), 2.55 (1H, bt, *J* = 11.7 Hz), 2.60-2.65 (2H, m), 2.77-2.82 (1H, m), 2.80 (1H, s), 2.90-2.94 (1H, m), 5.74 (1H, s), 6.56 (1H, d, *J* = 10.1 Hz), 6.66 (1H, d, *J* = 10.2 Hz) ppm
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	151 MHz
	Solvent:	MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 11.5, 23.1, 23.9, 25.2, 28.4, 32.6, 37.5, 39.0, 40.2, 50.4, 52.9, 73.8, 79.1, 89.2, 123.7, 125.6, 128.0, 142.6, 144.3, 159.9, 201.9 ppm
Melting point:		149-151 °C
Microanalysis:	Found:	C = 81.7%; H = 7.5% (August 2008)
	Calculated:	C = 81.8%; H = 7.8% (Calculated for $C_{21}H_{24}O_2$)