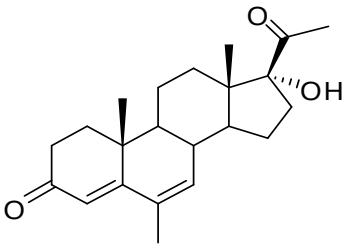
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D651.2015.01 (Bottled 160510)
This batch of bottles was prepared from the bulk material on 10th May 2016.

Compound Name: **Megestrol**
Collection Number: D651
Chemical Formula: $C_{22}H_{30}O_3$
CAS Registry Number: 3562-63-8
Structure:

Description: Pale yellow crystals
Batch Number: 00-AV-03
Molecular Weight: 342.5
Release Date: May 2000



Synonym: 17α-Hydroxy-6-methyl-4, 6-pregnadien-3,20-dione

Purity (mass fraction): 99.0 ± 1.8% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

HPLC:	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Methanol/water (75:25)
	Flow Rate:	1.0 mL/min
	Detector:	PDA at 293nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.0%, s = 0.11% (6 sub samples in duplicate, March 2010)
	Re-analysis:	Mean = 98.9%, s = 0.13% (5 sub samples in duplicate, March 2015)
HPLC:	Column:	Waters Nova Pak C-18, 5 μm (3.9 mm × 150 mm)
	Mobile Phase:	Methanol/water (70:30)
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.12% (10 sub samples in duplicate, August 2000)
	Re-analysis:	Mean = 99.9%, s = 0.04% (3 sub samples in duplicate, March 2005)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2 % mass fraction (September 2000 and April 2005)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (March, 2010) Moisture content < 0.1% mass fraction (March, 2015)

Spectroscopic and other characterisation data

GC-MS: *Tris*-trimethylsilyl derivative:

Instrument:	HP 6890/5973	
Column:	HP Ultra 1, 17 m × 0.20 mm I.D. × 0.11 μm	
Program:	170 °C (0.5 min), 10 °C /min to 300 °C (3 min)	
Injector:	260 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1 mL/min	Split ratio: 40/1

The retention time of the *tris*-TMS derivative is reported with the major peaks observed in the mass spectrum. The latter are reported in mass/charge ratios and (in brackets) as peak percentage relative to the intensity of the base peak.
Tris-TMS (9.8 min): 558 (M+, 16), 453 (16), 231 (25), 147 (17), 73 (100) m/z

ESI-MS:	Instrument:	Finnigan TSQ-700
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH_4OAc, pH 4.2: MeOH (1:1)
	Scan:	Scan range m/z 50-600, spray voltage: 4.5 kV
	Major ions:	343 (100, $[MH]^+$) m/z
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH_4OAc, pH 4.2: MeOH (1:1)
	Scan:	Scan range m/z 50-600, spray voltage: 3.0 kV.
	Major ions:	401 (100, $[M+CH_3COO]^-$), 387 (20, $[M+45]^-$), 341 (2, $[M-H]^-$) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/Ethyl acetate (4:1) Single spot observed, R_f = 0.29 (5 samples)
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, KBr disc
	Peaks:	3495, 1703, 1645, 1623, 1576, 1275, 1239, 888 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Key spectral data:	δ 0.79 (3H, s), 1.09 (3H, s), 1.84 (3H, s), 2.28 (3H, s), 5.83 (1H,s), 5.98 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz Solvent: $CDCl_3$ (76.9 ppm)
	Spectral data:	δ 15.3, 16.4, 19.8, 20.2, 23.4, 27.8, 30.2, 33.6, 33.7, 34.1, 36.1, 37.0, 47.9, 48.9, 50.4, 89.6, 121.2, 131.3, 138.3, 164.2, 199.9, 211.1 ppm
Melting point:		200-205 °C
Microanalysis:		Found: C = 77.2%; H = 8.6% (August, 2000) Calc: C = 77.2%; H = 8.8% (Calculated for $C_{22}H_{30}O_3$)

Expiration of certification
The property values are valid till 20th March 2020, 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.

The long-term stability of the compound in solution has not been examined.

This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

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 homogenous 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.

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.

Intended use
For ***in vitro*** laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

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Dr Stephen R. Davies,
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Dated: 11 May, 2016.