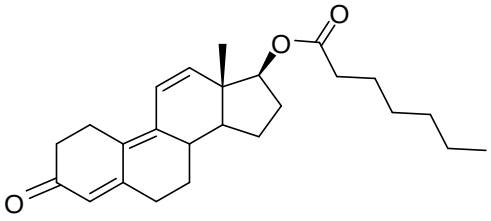
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: S028.2018.01 (Bottled 160822)
This batch of bottles was prepared from the bulk material on 22nd August 2016.

Compound Name: **Trenbolone enanthate**
Collection Number: S028
Chemical Formula: $C_{25}H_{34}O_3$
CAS Number: NA
Structure:

Description: Light yellow solid
Batch Number: 14-S-05
Molecular Weight: 382.5
Release date: 1st September 2014



Synonyms: 17β-Hydroxy-estra-4,9,11-trien-3-one heptanoate
17β-Hydroxyestra-4,9,11-trien-3-one 17-heptanoate

Purity (mass fraction): 91.8 ± 1.8% (95% coverage interval)

The purity value was obtained by quantitative nuclear magnetic resonance (qNMR). A combination of the one-proton doublet of doublets at 4.81 ppm and the one proton singlet at 5.78 ppm against a certified internal standard of dimethyl terephthalate. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent and elemental microanalysis.

HPLC:	Instrument:	Shimadzu or Waters Binary pump
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (90:10)
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A or Waters PDA 2998 operating at 340 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 96.8%, s = 0.02% (10 sub samples in duplicate, June 2014)
	Re-analysis:	Mean = 98.0%, s = 0.04% (5 sub samples in duplicate, July 2015)
	Re-analysis:	Mean = 97.9%, s = 0.03% (5 sub samples in duplicate, June 2016)
	Re-analysis:	Mean = 98.1%, s = 0.02% (5 sub samples in duplicate, July 2017)
	Re-analysis:	Mean = 98.1%, s = 0.02% (5 sub samples in duplicate, September 2018)
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (June 2014)
Karl Fischer analysis:		Moisture content ≤0.1% mass fraction (June 2014, 2015 and 2016) Moisture content 0.2% mass fraction (May 2017) Moisture content 0.2% mass fraction (May 2018)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Internal standard:	Dimethyl terephthalate (100% mass fraction)
	Initial analysis:	Mean (4.81 ppm) = 91.8%, s = 0.2% (3 sub samples, July 2014)
	Initial analysis:	Mean (5.78 ppm) = 91.8%, s = 0.4% (3 sub samples, July 2014)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro	
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)	
	Column temp:	40 °C	
	Solvent system:	2% Formic acid in water [1%], methanol [90% v/v], water [9% v/v]	
	Flow rate:	0.2 mL/min	
	Sample prep:	50 μg/g in MeOH/water (25:75)	
	Injection volume:	10 μL	
	Ionisation mode:	Electrospray positive ion	
	Capillary voltage:	3.5 kV	Cone voltage: 8 V
	Source temp:	130 ºC	Desolvation gas temperature: 350 ºC
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate: 745 L/hr
	The retention time of trenbolone enathate is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	5.9 min:	383 ($M+H^+$) *m/z*	
GC-MS:	Instrument:	Agilent 6890/5973	
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm	
	Program:	250 °C (1 min), 30 °C/min to 300 °C (10 min)	
	Injector:	250 °C	Transfer line temp: 280 °C
	Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1
	The retention time of the parent compound is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.		
	Parent (8.4 min):	382 (M^+, 16), 270 (38), 252 (100), 214 (18), 213 (17), 141 (9), 113 (12), 85 (8), 55 (8) *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:	Hexanes	
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/acetone (1/1) Single spot observed, R_f = 0.78. Visualisation with UV at 254 nm	
IR:	Instrument:	Biorad FTS3000MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	2951, 2932, 2858, 1731, 1654, 1585, 1572, 1377, 1238, 1172, 1100, 1016, 993, 888, 785, 773, 686 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	Solvent: $CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.89 (3H, t, *J* = 6.9 Hz), 0.94 (3H, s), 1.25-1.36 (6H, m), 1.44-1.77 (7H, m), 1.89 (1H, m), 2.27 (1H, m), 2.33 (2H, t, *J* = 7.5 Hz), 2.39-2.48 (3H, m), 2.50-2.62 (2H, m), 2.81 (2H, m), 4.81 (1H, dd, *J* = 7.4, 9.3 Hz), 5.78 (1H, s), 6.36 (1H, d, *J* = 9.9 Hz), 6.43 (1H, d, *J* = 9.9 Hz) ppm	
^{13}C NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	126 MHz	Solvent: $CDCl_3$ (77.2 ppm)
	Spectral data:	δ 14.2, 14.6, 22.7, 23.3, 24.5, 25.2, 27.1, 27.7, 29.0, 31.57, 31.62, 34.7, 36.8, 37.7, 45.2, 47.8, 78.3, 123.8, 124.0, 127.5, 141.6, 141.9, 156.4, 174.1, 199.4 ppm	
Melting point:		73-75 °C	
Microanalysis:		Found: C = 77.4%; H = 8.8% (June, 2014) Calc: C = 78.5%; H = 9.0% (Calculated for $C_{25}H_{34}O_3$)	

Expiration of certification
The property values are valid till 4th September 2021, i.e. three 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 three 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 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.

Metrological traceability
The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis. 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).

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
This certified reference material may be used for instrument calibration.

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:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 10 September, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 10th September 2018

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