Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

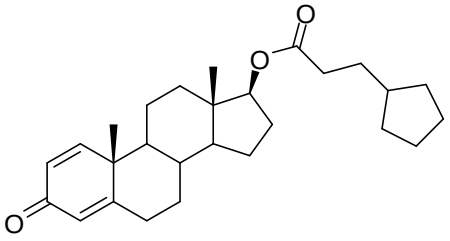
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S032: Boldenone cypionate

Report ID: S032.2017.02

Chemical Formula: $C_{27}H_{38}O_3$

Molecular Weight: 410.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
15-S-07	106505-90-2	97.3 ± 2.0%

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

IUPAC name: (17β)-3-Oxoandrosta-1,4-dien-17-yl 3-cyclopentylpropanoate.

Expiration of certification: The property values are valid till 28 July 2020, 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.

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:** In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years. 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.
21 April 2020

This report supersedes any issued prior to 21 April 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The purity value was obtained by quantitative nuclear magnetic resonance (qNMR). A combination of the one-proton doublet at 7.17 ppm, and the combined the combined one-proton triplet at 5.98 ppm plus the one proton doublet at 6.10 ppm were measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis, and ^{1}H NMR spectroscopy, headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40°C
	Mobile Phase:	Acetonitrile/MilliQ water (85:15 or 80:20)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 or Shimadzu SPD-M20A PDA operating at Max plot/244 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.8%, s = 0.02% (10 sub samples in duplicate, August 2015)
	Re-analysis:	Mean = 99.0%, s = 0.01% (5 sub samples in duplicate, August 2016)
	Re-analysis:	Mean = 99.0%, s = 0.03% (5 sub samples, in duplicate, July 2017)
Karl Fischer analysis:		Moisture content ca < 0.2% mass fraction (July 2015, 2016 and 2017)
Thermogravimetric analysis:		Volatile content 0.2% and non-volatile residue < 0.2% mass fraction (July 2015)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethylterephthalate (100.0% mass fraction)
	Initial analysis:	Mean (5.98 & 6.0 ppm) = 97.2%, s = 0.5% (5 sub samples, September 2015)
	Re-analysis:	Mean (7.17 ppm) = 97.4%, s = 0.05% (5 sub samples, September 2015)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	HP-1MS 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	250 °C (1 min), 10 °C/min to 300 °C (15 min)
	Injector:	250 °C, Split injection
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	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 (13.1 min):	410 (M^+, 2), 269 (4), 268 (4), 147 (28), 134 (11), 133 (16), 122 (100) 107 (13), 91 (13), 81 (10), 79 (11), 55 (21) *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:	Benzene, methyl cypionate
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (7:3) Single spot observed, R_f = 0.5. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	2937, 2926, 2906, 2847, 1726, 1665, 1626, 1451, 1308, 1238, 1200, 1117, 1016, 934, 890 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Key spectral data:	δ 0.86 (3H, s), 0.97-1.14 (5H, m), 1.19 (1H, dt, *J* = 4.2, 12.9 Hz), 1.23 (3H, s), 1.37 (1H, m), 1.44-1.87 (15H, m), 1.95 (1H, m), 2.18 (1H, m), 2.27-2.33 (2H, m), 2.36 (1H, ddd, *J* = 2.5, 4.2, 13.3 Hz), 2.46 (1H, ddd, *J* = 1.4, 5.0, 13.4 Hz), 4.58 (1H, dd, *J* = 7.9, 9.1 Hz), 6.07 (1H, t, *J* = 1.5 Hz), 6.22 (1H, dd, *J* = 1.9, 10.1 Hz), 7.04 (1H, d, *J* = 10.1 Hz) ppm
		Benzene estimated at < 0.1% and methyl cypionate estimated at 0.2% mass fraction was observed in the ^{1}H NMR.
^{13}C NMR	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.2 ppm)
	Spectral data:	δ 12.3, 18.9, 22.5, 23.8, 25.3, 27.6, 31.4, 32.6, 32.9, 33.2, 34.0, 35.5, 36.7, 39.8, 42.9, 43.7, 50.0, 52.4, 82.2, 124.1, 127.7, 155.9, 169.1, 174.1, 186.5 ppm
Melting point:		103-105 ºC
Microanalysis:	Found:	C = 78.8%; H = 9.6% (August 2015)
	Calculated:	C = 79.0%; H = 9.3% (Calculated for $C_{27}H_{38}O_3$)