Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

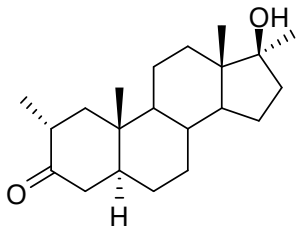
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S021: Methasterone

Report ID: S021.2018.02 (Bottled 150430)

Chemical Formula: $C_{21}H_{34}O_2$

Molecular Weight: 318.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
13-S-05	**3381-88-2**	**97.6 ± 1.1%**

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

IUPAC name: (2α,5α,17β)-17-Hydroxy-2,17-dimethylandrostan-3-one.

Expiration of certification: The property values are valid till 4 January 2023, 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 powder 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 2β-epimer is being formed when dissolved in acetonitrile and analysed by GC-FID.

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.
27 April 2020

This report supersedes any issued prior to 17 February 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 qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Agilent 7890A
	Column:	TG-17, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	250 °C (30 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 96.2%, s = 0.22% (10 sub samples in duplicate, January 2014)
GC-FID:	Instrument:	Varian CP3800
	Column:	TG-17 or HP-5, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	150 °C (1 min), 10 °C/min to 230 °C (10 min), 30 °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:	
	Re-analysis:	Mean = 97.8%, s = 0.03% (5 sub samples in duplicate, February 2017)
	Re-analysis:	Mean = 97.8%, s = 0.1% (5 sub samples in duplicate, January 2018)
Thermogravimetric analysis:		Volatile content 0.3% and non-volatile residue < 0.2% mass fraction (July 2013)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (January 2014, 2015, February 2016 & March 2017) Moisture content 0.2% mass fraction (February 2018)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m × 0.22 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	Bis-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m × 0.22 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the parent compound and *bis*-TMS derivative are reported along 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 (9.45 min):	318 (M^+, 32), 303 (70), 300 (30), 285 (100), 261 (78), 260 (49), 245 (85), 229 (37), 218 (25), 177 (48), 175 (33), 173 (28), 163 (47), 161 (37), 147 (32), 137 (55), 136 (32), 135 (34), 123 (65), 121 (37), 119 (38), 109 (33), 107 (56), 105 (49), 95 (38), 93 (58), 91 (48), 81 (74), 79 (56), 77 (38), 71 (52), 69 (38), 67 (65), 55 (37), 43 (74), 41 (45) *m/z*
	Bis-TMS (13.5 min):	462 (M^+, 58), 157 (12), 143 (100), 141 (30), 75 (26), 73 (55) *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:	Ethyl acetate
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (4/1) Single spot observed, R_f = 0.20. Visualisation with vanillin
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3509, 2961, 2936, 2880, 2919, 2851, 1702, 1444, 1364, 1169, 1077, 938, cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz Solvent: CD_3OD (3.31 ppm)
	Spectral data:	δ 0.73 (1H, m), 0.87 (3H, s), 0.84-0.95 (1H, m), 0.97 (3H, d, *J* = 6.8 Hz), 1.07 (1H, t, *J* = 13.0 Hz), 1.13 (3H, s), 1.18 (3H, s), 1.22-1.68 (12H, m), 1.74 (1H, m), 1.84 (1H, m), 1.98 (1H, dd, *J* = 3.5, 14.0 Hz), 2.11 (1H, dd, *J* = 6.0, 13.0 Hz), 2.43 (1H, ddd, *J* = 0.8, 13.9, 13.9 Hz), 2.57 (1H, m) ppm
		Ethyl acetate estimated at 0.2% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	101 MHz Solvent: CD_3OD (49.0 ppm)
	Spectral data:	δ 12.8, 14.7, 15.0, 22.3, 24.3, 26.2, 29.8, 32.7, 32.9, 37.4, 37.7, 39.2, 42.1, 45.6, 46.8, 49.6, 49.9, 51.9, 55.3, 82.2, 215.4 ppm
Melting point:		146-150 °C
Microanalysis:	Found:	C = 79.3%; H = 10.9% (July, 2013)
	Calculated:	C = 79.2%; H = 10.8% (Calculated for $C_{21}H_{34}O_2$)