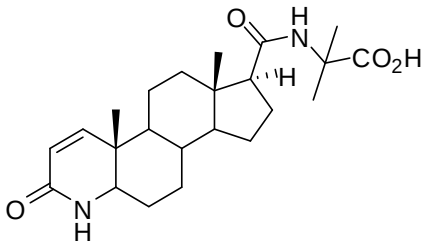
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S045: Carboxy Finasteride



Report ID: S045.2019.01

Chemical Formula: $C_{23}H_{34}N_2O_4$

Molecular Weight: 402.5g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
17-S-07	116285-37-1	81.5 ± 2.9%

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

IUPAC name: **N-{[(4aR,4bS,6aS,7S,9aS,9bS,11aR)-4a,6a-Dimethyl-2-oxo-2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11,11a-tetradecahydro-1H-indeno[5,4-f]quinolin-7-yl]carbonyl}-2-methylalanine**

Expiration of certification: The property values are valid till 14 January 2022, 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 material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: 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%.

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 seven 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 March 2019

This report supersedes any issued prior to 27 March 2019.

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 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 HPLC with UV detection, 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.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosample
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-13 min 24% B; 13-20 min 24-80% B; 20-23 min 80%B; 23-24 min 80-24%B, 24-32 min 24% B. Both aqueous and organic phases contained 0.05 % formic acid (v/v)
	Flow rate:	1 mL/min
	Detector:	Waters 2998 PDA operating at 208 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 89.9%, s = 0.2% (7 sub samples in duplicate, January 2018)
	Re-analysis:	Mean = 91.0%, s = 0.4% (5 sub samples in duplicate, January 2019)
Thermogravimetric analysis:		Volatile content 8.1% and non volatile residue 0.2 – 0.3 % mass fraction (April 2018).
Karl Fischer analysis:		Moisture content 8.1% mass fraction (January 2018) Moisture content 9.0% mass fraction (January 2019)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector
	Column:	X-Bridge C-18, 100 mm × 2.1 mm I.D. × 3.5 µm
	Column temp:	40 °C
	Solvent system:	A = MilliQ water; B = Acetonitrile 0-13 min 24% B; 13-20 min 24-80% B; 20-23 min 80%B; 23-24 min 80-24%B, 24-32 min 24% B Both aqueous and organic phases contained 0.05 % formic acid (v/v)
	Flow rate:	0.2 mL/min
	Sample prep:	100 µg/g in mobile phase
	Injection volume:	10 µL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	3.5 kV
	Cone voltage:	17 V
	Source temp:	120 ºC
	Desolvation gas temp:	350 ºC
	Cone gas flow rate:	1 L/hr
	Desolvation gas flow:	600 L/hr
	The retention time of carboxy finasteride is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	4.3 min:	403.2 ($M+H^+$) *m/z*
ESI-MS:	Instrument:	Micromass Quattro Micro
	Operation:	Negative ion mode, direction infusion at 5.0 µL/min
	Ionisation:	ESI spray voltage at 3.00 kV negative ion
	EM voltage:	650 V
	Cone voltage:	30 V
	Peak:	401.1 ($M-H^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Dichloromethane/methanol (85/15)
	Single spot observed,	R_f = 0.31 (streaks)
	Visualisation with UV at 254 nm and permanganate.	
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3577, 3470, 3220, 3171, 3049, 2967, 2933, 2865, 2840, 1722, 1643, 1594, 1543, 1272, 1165, 813 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.71 (s, 3H), 0.96 (s, 3H), 1.05-1.10 (m, 2H), 1.17-1.22 (m, 1H), 1.26-1.64 (m, 12H), 1.66-1.74 (m, 3H), 1.77-1.84 (m, 2H), 1.97-2.01 (m, 1H), 2.15 (dd, 1H, *J* =10.8, 20.0 Hz), 2.26 (t, 1H, *J* = 9.3 Hz), 3.33-3.35 (m, 1H), 5.76 (d, 1H, *J* = 9.9 Hz), 6.97 (d, 1H, *J* = 9.9 Hz), 7.92 (s, 1H) ppm Both dichloromethane and acetone were measured at < 0.1% mass fraction respectively.
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	151MHz Solvent: MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 12.2, 13.9, 22.2, 24.4, 25.3, 25.4, 25.7, 26.3, 30.7, 36.6, 38.8, 40.4, 45.9, 56.86, 56.94, 57.0, 60.9, 123.1, 153.6, 168.8, 174.5, 178.2 ppm
Microanalysis:	Found:	C = 62.5%; H = 9.0%; N = 6.4% (January 2018)
	Calc:	C = 63.0%; H = 8.7%; N = 6.4% (Calculated for $C_{23}H_{34}N_2O_4.2H_2O$)