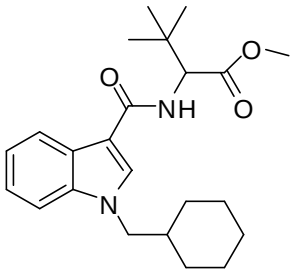
Australian Government
Department of Industry, Science, Energy and Resources
National Measurement Institute
NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1054: Methyl 2-[[1-(cyclohexylmethyl)-1H-indol-3-yl]formamido]-3,3-dimethylbutanoate



Report ID: D1054.2022.01

Chemical Formula: $C_{23}H_{32}N_2O_3$

Molecular Weight: 384.5 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
15-D-10	1971007-95-0 (for L-isomer)	99.2 ± 0.3%

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

IUPAC name: Methyl N-{[1-(cyclohexylmethyl)-1H-indol-3-yl]carbonyl}-3-methyl-valinate.

Expiration of certification: The property values are valid till 20 May 2027, 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 25 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 ten 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.
28 June 2022

This report supersedes any issued prior to 28 June 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (70:30 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 294 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (10 sub samples in duplicate, September 2015)
	Re-analysis:	Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, September 2016)
	Re-analysis:	Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, August 2017)
	Re-analysis:	Mean = 99.9%, s = 0.004% (5 sub samples in duplicate, August 2022)
Karl Fischer analysis:		Moisture content 0.2% mass fraction (September 2015, August 2016, August 2017, July 2018 and April 2022)
Thermogravimetric analysis:		Volatiles content 0.6 % and non-volatile residue < 0.2% mass fraction (December 2015)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 µm
	Program:	180 °C (1 min), 20 °C/min to 300 °C (10 min)
	Injector:	250 °C,
	Split ratio:	20/1
	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 compound (9.67 min):	384 (M^+, 16), 328 (19), 245 (16), 240 (100), 144 (26) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (3:1) Single spot observed, R_f = 0.4
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	2925, 2852, 1742, 1732, 1613, 1534, 1509, 1451, 1239, 1196, 1153, 1128, 738, 617 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.96-1.07 (2H, m), 1.03 (9H, s), 1.13-1.23 (3H, m), 1.45-1.73 (5H, m), 1.82 (1H, m), 3.66 (3H, s), 4.04 (2H, m), 4.49 (1H, d, *J* = 8.7 Hz), 7.12 (1H, dt, *J* = 0.6, 7.9 Hz), 7.18 (1H, dt, *J* = 1.0, 8.0 Hz), 7.53 (1H, d, *J* = 8.0 Hz), 7.64 (1H, d, *J* = 8.6 Hz), 8.09 (1H, d, *J* = 7.9 Hz), 8.36 (1H, s) ppm
	Hexane estimated at 0.5% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 25.2, 25.9, 26.8, 30.2, 30.3, 33.9, 51.5, 52.1, 60.0, 108.6, 110.6, 120.7, 121.2, 122.0, 126.6, 132.3, 136.5, 164.5, 172.1 ppm
Melting point:		135-136 °C
Microanalysis:	Found:	C = 71.6%; H = 8.4%; N = 7.3% (September, 2015)
	Calculated:	C = 71.8%; H = 8.4%; N = 7.3% (Calculated for $C_{23}H_{32}N_2O_3$)