Australian Government
Department of Industry, Science and Resources

National Measurement Institute

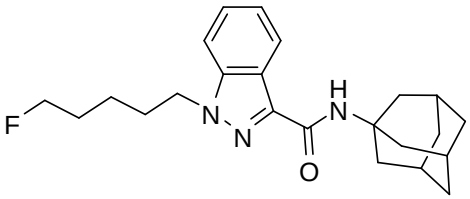
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D1020: N-(1-Adamantyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide

Report ID: D1020.2020.03

Chemical Formula: $C_{23}H_{30}FN_3O$

Molecular Weight: 383.5 g/mol (HCl)




## Certified value

| Batch No. | CAS No. | Purity (mass fraction) |
|---|---|---|
| **14-D-19** | **1400742-13-3** | **98.7 ± 0.3%** |

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

**IUPAC name:** *N*-(1-Adamantyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide.

**Expiration of certification:** The property values are valid till 29 May 2025, 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:** 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 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 September 2022

This report supersedes any issued prior to 21 September 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 elemental microanalysis.

| | | |
|---|---|---|
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters 2695 Separation module |
| | Column: | Alltima C-18, 5 μm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | A = MilliQ water; B = Acetonitrile<br>0-13 min 70% B, 13-14 min 70-90% B, 14-19 min 90% B, 19-20 min 90-70% B, 20-28 min 70% B |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Shimadzu SPD-M20A or Waters 2998 PDA operating at 300 nm |
| | Relative mass fraction of the main component: | |
| | Initial analysis: | Mean = 98.7%, s = 0.05% (10 sub samples in duplicate, June 2014) |
| | Re-analysis: | Mean = 98.7%, s = 0.05% (5 sub samples in duplicate, June 2015) |
| | Re-analysis: | Mean = 98.8%, s = 0.06% (5 sub samples in duplicate, June 2016) |
| | Re-analysis: | Mean = 98.5%, s = 0.06% (5 sub samples in duplicate, June 2017) |
| | Re-analysis: | Mean = 98.8%, s = 0.02% (5 sub samples in duplicate, May 2020) |
| Karl Fischer analysis: | | Moisture content ≤ 0.1% mass fraction (June 2014, 2015, 2016, 2017 and May 2020) |
| Thermogravimetric analysis: | | Non-volatile residue < 0.2% mass fraction. The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis. (June 2015) |

## Spectroscopic and other characterisation data

| | | | | |
|---|---|---|---|---|
| GC-MS: | Instrument: | HP6890/5973 | | |
| | Column: | TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm | | |
| | Program: | 180 °C (1 min), 30 °C/min to 300 °C (20 min) | | |
| | Injector: | 250 °C | | |
| | Split ratio: | 30/1 | | |
| | Transfer line temp: | 280 °C | | |
| | Carrier: | Helium | | |
| | 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 (9.65 min): | 383 ($M^+$, 50), 355 (61), 338 (22), 326 (18), 294 (54), 233 (100), 150 (32), 145 (49), 135 (14), 131 (15) *m/z* | | |
| LC-MS: | Instrument: | Waters Alliance/ Micromass Quattro TQ Detector | | |
| | Column: | Alltima C-18, 5 μm (4.6 mm x 150 mm) | | |
| | Column temp: | 40°C | | |
| | Solvent system: | MilliQ water containing 2% formic acid [5% v/v], acetonitrile [80% v/v], MilliQ water [15% v/v] | | |
| | Flow rate: | 0.2 mL/min | | |
| | Sample prep: | 1 μg/g in mobile phase (25:75) | | |
| | Injection volume: | 10 μL | | |
| | Ionisation mode: | Electrospray positive ion | | |
| | Capillary voltage: | 3.5 kV | Cone voltage: | 20 V |
| | Source temp: | 130 ºC | Desolvation gas temperature: | 350 ºC |
| | Cone gas flow rate: | 23 L/hr | Desolvation gas flow rate: | 753 L/hr |
| | The retention time of 5-fluoro-AKB48 is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio. | | | |
| | 6.4 min: | 384.3 ($M+H^+$) *m/z* | | |
| TLC: | Conditions: | Kieselgel 60$F_{254}$. Hexane/ethyl acetate (4:1)<br>Single spot observed, $R_f$ = 0.5 | | |
| IR: | Instrument: | Biorad FTS3000MX FT-IR | | |
| | Range: | 4000-400 $cm^{-1}$, KBr powder | | |
| | Peaks: | 3296, 3058, 2905, 2848, 1651, 1536, 1492, 1455, 1358, 1292, 1206, 1182, 997, 860, 753 $cm^{-1}$ | | |
| $^1$H NMR: | Instrument: | Bruker Ascend-500 | | |
| | Field strength: | 500 MHz | | |
| | Solvent: | DMSO-$d_6$ (2.50 ppm) | | |
| | Spectral data: | δ 1.33 (2H, m), 1.65 (2H, m), 1.67 (6H, bs), 1.86 (2H, m), 2.07 (3H, bs), 2.10 (6H, bs), 4.35 (1H, t, *J* = 6.0 Hz), 4.45 (1H, t, *J* = 6.0 Hz), 4.47 (2H, t, *J* = 7.0 Hz), 7.13 (1H, s), 7.25 (1H, ddd, *J* = 0.7, 6.9, 8.0 Hz), 7.44 (1H, ddd, *J* = 0.7, 6.9, 8.0 Hz), 7.75 (1H, dt, *J* = 8.7, 0.9 Hz), 8.14 (1H, dt, *J* = 8.3, 1.0 Hz) ppm | | |
| $^{13}$C NMR: | Instrument: | Bruker Ascend-500 | | |
| | Field strength: | 126 MHz | | |
| | Solvent: | DMSO-$d_6$ (39.52 ppm) | | |
| | Spectral data: | δ 22.1 ($J_{CF}$ = 5.3 Hz), 28.9, 29.1, 29.4 ($J_{CF}$ = 19.3 Hz), 36.0, 41.1, 48.4, 51.1, 83.7 ($J_{CF}$ = 161.7 Hz) , 110.4, 121.9, 122.0, 122.3, 126.6, 137.5, 140.6, 161.1 ppm | | |
| $^{19}$F NMR: | Instrument: | Bruker Ascend 500 | | |
| | Field strength: | 470 MHz | | |
| | Solvent: | DMSO-$d_6$ | | |
| | Spectral data: | δ -217.0 ppm | | |
| Melting point: | | 61-67 °C | | |
| Microanalysis: | Found: | C = 72.1%; H = 8.1%; N = 11.1%; Cl = 5.0% (June, 2014) | | |
| | Calculated: | C = 72.0%; H = 7.9%; N = 11.0%; Cl = 5.0% (Calculated for $C_{23}H_{30}FN_3O$) | | |