Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

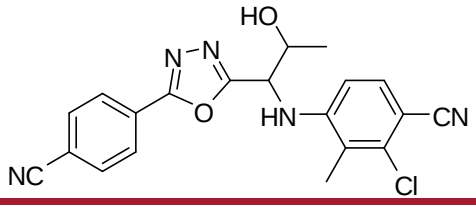
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D1076: RAD-140

Report ID: D1076.2023.01

Chemical Formula: $C_{20}H_{16}ClN_5O_2$

Molecular Weight: 393.8 g/mol



Property value

Batch No.	CAS No.	Purity estimate
20-D-05	**1182367-47-0**	**89.6 ± 2.4%**

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

IUPAC name: 2-Chloro-4-(1-[5-(4-cyanophenyl)-1,3,4-oxadiazol-2-yl]-2-hydroxypropylamino)-3-methylbenzonitrile

The stereochemistry at C1 and C2 of the 2-hydroxypropylamino chain is reported as (1R, 2S) in the literature (Miller, C. P., Shomali, M., Lyttle C. R. *et al* ACS Med. Chem., Lett., **2011**, 2, 124-129). The relative stereochemistry (1RS, 2SR) of the main component has been established by comparison with literature ^{1}H and ^{13}C NMR spectra in $CDCl_3$. The enantiomeric purity of the material has not been established.

Expiration of certification: The property values are valid till 22 June 2026, 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 powder sourced from an external supplier, then certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap.

Intended use: This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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.

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 measurement uncertainty at the 95% confidence interval also includes a stability component estimated from an accelerated stability trial, conducted at 40 ºC and 75% humidity for a 14 day period.

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.
26 June 2023

This report supersedes any issued prior to 26 June 2023.

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

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 purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include 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

The purity value obtained by quantitative nuclear magnetic resonance (qNMR) used a combination of the one-proton doublet at 6.79 ppm, the one-proton quartet at 5.12 ppm and the one-proton multiplet at 4.41 ppm measured against a certified internal standard of *bis*-trimethylsilylbenzene.

Supporting evidence is provided by elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	*Bis*-Trimethylsilylbenzene (100.0% mass fraction)
	Initial analysis:	Mean (6.79 ppm) = 89.4%, s = 0.3% (5 sub samples, August 2020)
	Initial analysis:	Mean (5.12 ppm) = 89.3%, s = 0.2% (5 sub samples, August 2020)
	Initial analysis:	Mean (4.41 ppm) = 89.4%, s = 0.2% (5 sub samples, August 2020)
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:	A = Milli-Q water; B = Acetonitrile 0-25 min 40% B; 25-30 min 40-80% B; 30-33min 80% B; 33-35 min 80-40% B, 35-50 40% B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 278 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 89.8%, s = 0.6% (7 sub samples in duplicate, August 2020)
HPLC:	Instrument:	Waters alliance 2695 separation module or Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler or Thermo Ulitmate 3000
	Column:	ACE Excel Super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water, B = Methanol, Isocratic 57%B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 or Shimadzu SPD-M20A or RS PDA operating at 278 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 90.7%, s = 0.3% (5 sub samples in duplicate, October 2021)
	Re-analysis:	Mean = 88.3%, s = 0.7% (5 sub samples in duplicate, August 2022)
	Re-analysis:	Mean = 89.7%, s = 0.4% (5 sub samples in duplicate, June 2023)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (August 2020) Moisture content 0.3% mass fraction (August 2021) Moisture content 0.3% mass fraction (August 2022) Moisture content ≤ 0.1% mass fraction (May 2023)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (September 2020)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector	
	Column:	Alltima C-18, 150 mm × 4.6 mm I.D. × 5 μm	
	Column temp:	40 °C	
	Solvent system:	A = 0.1 percent formic acid; B = Acetonitrile 0-20 min 40% B; 20-25 min 40-80% B; 25-28 min 40%B; 28-30 min 80-40%B.	
	Flow rate:	0.2 mL/min	
	Sample prep:	1000 μg/g in mobile phase	
	Injection volume:	30 μL	
	Ionisation mode:	Electrospray negative ion	
	Capillary voltage:	2.5 kV	Cone voltage: 20 V
	Source temp:	120 ºC	Desolvation gas temperature: 400 ºC
	Cone gas flow rate:	23 L/hr	Desolvation gas flow rate: 600 L/hr
	The retention time of RAD-140 is reported along with the major peaks in the mass spectrum. The latter is reported as a mass/charge ratio.		
	19.5 min:	396.1 $[MCl^{37}+H]^+$, 394.1 $[MCl^{35}+H]^+$, 225.1 $[MCl^{37}\text{-}NCC_6H_4C_2N_2O+H]^+$, 223.1 $[MCl^{35}\text{-}NCC_6H_4C_2N_2O+H]^+$ *m/z*	
IR:	Instrument:	Bruker Alpha Platinum ATR	
	Range:	4000-400 cm^{-1}, neat	
	Peaks:	3449, 2227, 1590, 1553, 1514, 1493, 1407, 1331, 1247, 1168, 1136, 1086, 1069, 1015, 849, 826, 739, 702, 551 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	
	Solvent:	$CDCl_3$ (7.26 ppm)	
	Spectral data:	δ 1.45 (3H, d, *J* = 6.3 Hz), 2.36 (3H, s), 2.99 (1H, bs), 4.62 (1H, dq, *J* = 2.7, 6.3 Hz), 4.79 (1H, dd, *J* = 2.7, 8.0 Hz), 5.27 (NH, bd, *J* = 8.0 Hz), 6.63 (1H, d, *J* = 8.5 Hz), 7.38 (1H, d, *J* = 8.5 Hz), 7.78 (2H, d, *J* = 8.7 Hz), 8.08 (2H, d, *J* = 8.7 Hz) ppm	
	Dichloromethane and ethyl acetate estimated at 0.03% and 0.02% mass fraction respectively were quantified by ^{1}H NMR.		
^{13}C NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	126 MHz	
	Solvent:	$CDCl_3$ (77.16 ppm)	
	Spectral data:	δ 13.9, 20.2, 54.9, 68.2, 102.5, 108.5, 115.8, 117.7, 117.8, 121.9, 127.2, 127.6, 132.9, 133.0, 137.2, 149.0, 164.0, 166.5 ppm	
Melting point:		183-185 °C	
Microanalysis:	Found:	C = 61.2%; H = 4.0%; N = 17.9% (June 2020)	
	Calculated:	C = 61.0%; H = 4.1%; N = 17.8% (Calculated for $C_{20}H_{16}ClN_5O_2$)	