Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

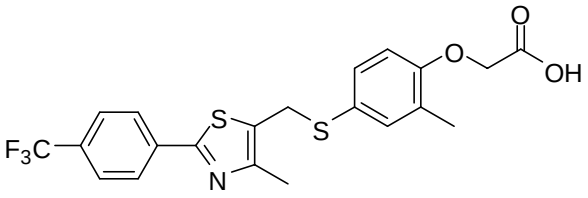
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1077: Cardarine

Report ID: D1077.2021.01

Chemical Formula: $C_{21}H_{18}F_3NO_3S_2$

Molecular Weight: 453.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
20-D-06	317318-70-0	98.3 ± 1.5%

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

IUPAC name: {2-Methyl-4-[({4-methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-5-yl}methyl)sulfanyl]phenoxy}acetic acid

Expiration of certification: The property values are valid till 17 August 2024, 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: 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.

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%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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. 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 short term accelerated stability trials and long term stability trials conducted on similar compounds. 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.
10 September 2021

This report supersedes any issued prior to 10 September 2021

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 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 quantitative NMR, headspace GC-MS analysis of occluded solvents, and elemental microanalysis. The purity value obtained by quantitative nuclear magnetic resonance (qNMR) used the one-proton doublet of doublets at 6.77 ppm measured against a certified internal standard of *bis*-trimethylsilylbenzene.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile / Milli-Q water with 0.2 % formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 315 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.7%, s = 0.03% (7 sub samples in duplicate, August 2020)
HPLC:	Instrument:	Waters alliance 2695
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water with 0.1 % formic acid; B = Acetonitrile 0-9 min 65% B; 9-11 min 65-85% B; 11-16 min 85%B; 16-17 min 85-65%B.
	Flow rate:	1.0 mL/min.
	Detector:	Waters 2998 PDA operating at 315 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.8%, s = 0.02% (5 sub samples in duplicate, August 2021)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (August 2020) Moisture content < 0.1% mass fraction (August 2021)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (September 2020)
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 (6.77 ppm) = 98.4%, s = 0.4% (5 sub samples, August 2020)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector	
	Column:	X-Bridge 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	
	Flow rate:	0.2 mL/min	
	Sample prep:	50 μg/g in mobile phase	
	Injection volume:	10 μL	
	Ionisation mode:	Electrospray positive ion	
	Capillary voltage:	2.5 kV	Cone voltage: 20 V
	Source temp:	120 ºC	Desolvation gas temperature: 400 ºC
	Cone gas flow rate:	Off	Desolvation gas flow rate: 600 L/hr
	The retention time of cardarine is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	8.4 min:	454.6 $[M+H]^+$ *m/z*	
IR:	Instrument:	Bruker Alpha Platinum ATR	
	Range:	4000-400 cm^{-1}, neat	
	Peaks:	2921, 1715, 1488, 1442, 1323, 1218, 1167, 1114, 1068, 1001, 841, 798, 679, 646, 602 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	
	Solvent:	$CDCl_3$ (7.26 ppm)	
	Spectral data:	δ 2.15 (3H, s), 2.23 (3H, s), 4.10 (2H, s), 4.67 (2H, s), 6.62 (1H, d, *J* = 8.5 Hz), 7.10 (1H, dd, *J* = 2.2, 8.5 Hz), 7.22 (1H, d, *J* = 1.6 Hz), 7.66 (2H, d, *J* = 8.3 Hz), 7.96 (2H, d, *J* = 8.1 Hz) ppm	
	Ethyl acetate estimated at 0.2% mass fraction was observed in the ^{1}H NMR.		
^{13}C NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	126 MHz	
	Solvent:	$CDCl_3$ (77.16 ppm)	
	Spectral data:	δ 14.7, 16.2, 32.5, 65.2, 111.7, 124.0 (q, *J* = 272.9 Hz), 125.6, 126.1 (q, *J* = 3.7 Hz), 126.7, 128.5, 131.0, 131.6 (q, *J* = 32.8 Hz), 132.4, 136.4, 136.6, 151.4, 156.3, 163.7, 172.6 ppm	
Melting point:		145-147 °C	
Microanalysis:	Found:	C = 55.7%; H = 3.8%; N = 3.1%; S% = 14.4% (June, 2020)	
	Calculated:	C = 55.6%; H = 4.0%; N = 3.1%; S% = 14.2% (Calculated for $C_{21}H_{18}F_3NO_3S_2$)	