Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

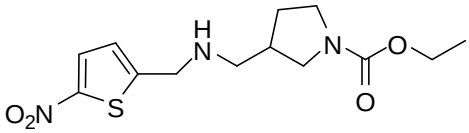
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1067: Ethyl N-(5-nitro-2-methylthiophene)-3-aminomethylpyrrolidine-1-carboxylate

Report ID: D1067.2020.01

Chemical Formula: $C_{13}H_{19}N_3O_4S$

Molecular Weight: 313.4 g/mol



Certified value

Batch No.	CAS No.	Purity estimate
16-D-06	**N/A**	**98.3 ± 0.5%**

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

Human metabolite of SR9009, reported as *metabolite M2* by researchers in Russia (T Sobolevsky, M Dikunets and G Rodchenkov, *Recent Advances in Doping Analysis*, **2015**, 23, 64-69).

Expiration of certification: The property values are valid till 28 April 2023, 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.

Description: Off-pink solid prepared by synthesis, 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: This material has demonstrated stability over a minimum period of 3 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.
20 May 2020

This report supersedes any issued prior to 20 May 2020.

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.

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	32 °C
	Mobile Phase:	20mM Ammonium Acetate buffer pH= 6.0/Acetonitrile A = Ammonium Acetate; B = Acetonitrile 0-10 min 40% B; 10-20 min 40-80% B; 20-25 min 80%B; 25-26 min 80-40%B; 26-30 min 40%B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A or Waters 2998 PDA operating at 334 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.1%, s = 0.01% (5 sub samples in duplicate, March 2018)
	Re-analysis:	Mean = 98.9%, s = 0.04% (5 sub samples in duplicate, March 2019)
	Re-analysis:	Mean = 98.8%, s = 0.02% (5 sub samples in duplicate, April 2020)
Karl Fischer analysis:		Moisture content ≤ 0.2% mass fraction (March 2017, 2019 and 2020)
Thermogravimetric analysis:		Non volatiles residue < 0.2% mass fraction (March 2017). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis.

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector
	Column:	Poroshell C-18, 100 mm × 2.1 mm I.D. × 3.5 μm
	Column temp:	Ambient
	Solvent system:	A = MilliQ water; B = Methanol 0-15 min 55% B; 15-20 min 55-80% B; 20-25 min 80%B; 25-27 min 80-55%B. The aqueous phase was buffered at pH 7.8 using 10mM NH_4HCO_2 and NH_3
	Flow rate:	0.3 mL/min
	Sample prep:	2000 μg/g in MeOH/pH 7.8 buffer (55:45)
	Injection volume:	10 μL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	1 kV
	Cone voltage:	17 V
	Source temp:	120 ºC
	Desolvation gas temp:	350 ºC
	Cone gas flow rate:	2 L/hr
	Desolvation gas flow:	600 L/hr
	The retention time of ethyl 3-[[[(5-nitro-2-thienyl)methyl]amino]methyl]-1-pyrrolidinecarboxylate is reported with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	Parent (4.40 min):	314.1 ($M+H^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (1/9) Single spot observed, R_f = 0.32. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3330, 1680, 1495, 1467, 1447, 1425, 1336, 1162, 1101, 832, 812 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH-*d*$_4$ (3.31ppm)
	Spectral data:	δ 1.23-1.27 (3H, m), 1.66 (1H, m), 2.06 (1H, m), 2.38 (1H, m), 2.61 (1H, m), 2.67 (1H, dd, *J* = 6.7, 11.5 Hz), 3.10 (1H, m), 3.32 (1H, m), 3.46 (1H, m), 3.57 (dd, *J* = 7.7, 10.0 Hz), 4.00 (2H, m), 4.10 (2H, q, *J* = 7.0 Hz), 7.01 (1H, d, *J* = 4.0 Hz), 7.87 (1H, d, *J* = 4.0 Hz) ppm
		Hexane estimated at 0.2% mass fraction and TBME at 0.1% mass fraction were observed in the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz
	Solvent:	MeOH-*d*$_4$ (49.0 ppm)
	Spectral data:	δ 15.1, [30.0, 30.8]*, [39.7, 40.4]*, [46.4, 46.6]*, 49.49, [50.9, 51.2]*, [52.69, 52.71]* 62.3, 125.4, 130.1, 151.6, 156.6, [157.0, 157.1]* ppm
		*Evidence of rotamers
Melting point:		86 °C (decomposition)
Microanalysis:	Found:	C = 49.9%; H = 6.2%; N = 13.5%; S = 10.2% (March 2017)
	Calculated:	C = 49.8%; H = 6.1%; N = 13.4%; S = 10.2% (Calculated for $C_{13}H_{19}N_3O_4S$)