



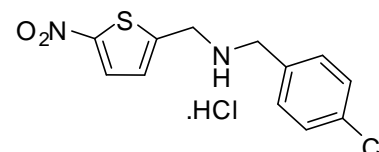
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D1066: N-[(4-Chlorophenyl)methyl]-5-nitro-2-thiophenemethanamine hydrochloride

Report ID: D1066.2019.01 (Ampouled 170810)

Chemical Formula: C₁₂H₁₁ClN₂O₂S.HCl

Molecular Weight: 319.2 g/mol (HCl), 282.8 g/mol (base)



Certified value

Batch No.	CAS No.	Mass per ampoule
16-D-05	1384516-10-2 (free base)	997 ± 18 µg

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

IUPAC name: 1-(4-Chlorophenyl)-N-[(5-nitro-2-thienyl)methyl]methanamine.

Expiration of certification: The property values are valid till 13 March 2022, 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 ampoules 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: The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D1066. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 997 ± 18 µg of anhydrous N-[(4-chlorophenyl)methyl]-5-nitro-2-thiophenemethanamine hydrochloride.

Recommended storage: When not in use, this material should be stored at or below 25 °C 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 10 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 five randomly selected ampoules 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 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.
22 March 2019

This report supersedes any issued prior to 22 March 2019

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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	32 °C
	Mobile Phase:	Milli Q water/Acetonitrile (0-3 min 45% B, 3-3.30 min 45%-55% B, 3.3-13 min 55% B, 13-13.30 min 55%-75% B, 13.3-20 min 75% B, 20-20.05 min 75%-45% B, 20.05-30 min 45% B) The aqueous phase was buffered at pH 6.0 using 20 mM ammonium acetate and acetic acid
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA or Waters 2998 PDA operating at 220 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.4%, s = 0.03% (6 ampoules in duplicate, March 2018)
	Re-analysis:	Mean = 99.5%, s = 0.02% (5 ampoules in duplicate, March 2019)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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 ¹H NMR spectroscopy. The purity value is calculated as per Equation 1

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

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:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	32 °C
	Mobile Phase:	Milli Q water/ Acetonitrile (0-3 min 45% B, 3-13 min 55% B, 13-20 min 75% B, 20-30 min 45% B) The aqueous phase was buffered at pH 6.0 using 20mM ammonium acetate and acetic acid
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 220 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.44%, s = 0.05% (8 sub samples in duplicate, February 2018)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (December 2016). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis
Karl Fischer analysis:		Moisture content 0.3% mass fraction (December 2016)

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:	Methanol/MilliQ water (65:35 v/v) The aqueous phase was buffered at pH 7.8 using 10 mM NH ₄ CO ₂ and NH ₃
	Flow rate:	0.3 mL/min
	Sample prep:	2000 µg/g in MeOH/MilliQ water (65:35)
	Injection volume:	5 µL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	1.0 kV Cone voltage: 1.0 V
	Source temp:	120 °C Desolvation gas temperature: 350 °C
	Cone gas flow rate:	1 L/hr Desolvation gas flow rate: 600 L/hr
	The retention time of N-[(4-chlorophenyl)methyl]-5-nitro-2-thiophenemethanamine is reported along with the major peaks in the mass spectrum. The latter is reported as a mass/charge ratio.	
	5.5 min:	282.9 (³⁵ M+H ⁺), 285.0 (³⁷ M+H ⁺) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate/diethyl amine (15/10/1) Single spot observed, R _f = 0.52. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm ⁻¹ , neat
	Peaks:	3099, 2931, 2911, 2715, 1510, 1433, 1340, 833, 812, 730, 642, 530, 485 cm ⁻¹
¹ H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz Solvent: MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 4.31 (2H, s), 4.57 (2H, s), 7.38 (1H, d, <i>J</i> = 4.0 Hz), 7.49 (2H, dt, <i>J</i> = 8.6, 2.1 Hz), 7.53 (2H, dt, <i>J</i> = 8.6, 2.2 Hz), 7.99 (1H, d, <i>J</i> = 4.0 Hz) ppm Ethanol estimated at 0.05% mass fraction was observed in the ¹ H NMR
¹³ C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz Solvent: MeOH- <i>d</i> ₄ (49.0 ppm)
	Spectral data:	δ 46.1, 51.5, 129.8, 130.5, 130.9, 131.9, 132.9, 137.0, 140.8, 154.6 ppm
Melting point:		> 200 °C (dec.)
Microanalysis:	Found:	C = 45.2%; H = 3.9%; N = 8.8%; Cl = 22.0%; S = 9.9% (December 2016)
	Calc:	C = 45.2%; H = 3.8%; N = 8.8%; Cl = 22.2%; S = 10.1% (Calculated for C ₁₂ H ₁₁ ClN ₂ O ₂ S.HCl)