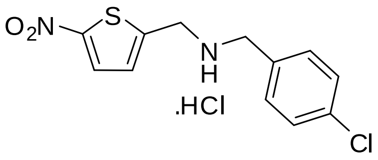
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

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



Report ID: D1066.2023.01 (Ampouled 170810)

Chemical Formula: $C_{12}H_{11}ClN_2O_2S.HCl$

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

Property value

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

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 hydrochloride

Expiration of certification: The property values are valid till 04 January 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D1066. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

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 approximately 997 ± 18 µg of anhydrous *N*-[(4-chlorophenyl)methyl]-5-nitro-2-thiophenemethanamine hydrochloride. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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: 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 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.
5 January 2023

This report supersedes any issued prior to 5 January 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:

HPLC:
Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler or Waters Model 1525 Binary pump, 717 plus auto sampler
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 mass fraction of the 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)
Re-analysis: Mean = 99.4%, s = 0.02% (5 ampoules in duplicate, April 2020)
Re-analysis: Mean = 99.3%, s = 0.02% (5 ampoules in duplicate, January 2023)

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

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

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:
Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler
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 20 mM ammonium acetate and acetic acid
Flow rate: 1 mL/min
Detector: Shimadzu SPD-M20A PDA operating at 220 nm

Relative mass fraction of the 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/Milli-Q water (65:35 v/v)
The aqueous phase was buffered at pH 7.8 using 10 mM NH_4CO_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 ºC Desolvation gas temperature: 350 ºC
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$^+$) *m/z*

TLC:
Conditions: Kieselgel 60F_{254}. Hexane/ethyl acetate/diethyl amine (15/10/1)
Single spot observed, R_f = 0.5. 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-d_4 (3.31ppm)
Spectral data: δ 4.31 (2H, s), 4.57 (2H, s), 7.38 (1H, d, J = 4.0 Hz), 7.49 (2H, dt, J = 8.6, 2.1 Hz), 7.53 (2H, dt, J = 8.6, 2.2 Hz), 7.99 (1H, d, J = 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-d_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)
Calculated: C = 45.2%; H = 3.8%; N = 8.8%; Cl = 22.2%; S = 10.1%
(Calculated for $C_{12}H_{11}ClN_2O_2S.HCl$)