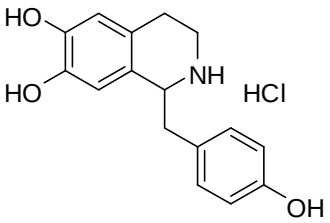
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1070: Higenamine hydrochloride



Report ID: D1070.2022.01

Chemical Formula: $C_{16}H_{17}NO_3.HCl$

Molecular Weight: 307.8 g/mol (HCl), 271.3 g/mol (HCl)

Certified value

Batch No.	CAS No.	Purity (mass fraction)
17-D-04	**11041-94-4 (HCl)** **5843-65-2 (base)**	**97.3 ± 0.5%**

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

IUPAC name: 1-(4-Hydroxybenzyl)-1,2,3,4-tetrahydro-6,7-isoquinolinediol hydrochloride.

Expiration of certification: The property values are valid till 1 September 2027, i.e. five 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-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 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 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: This material has demonstrated stability over a minimum period of five 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.
11 November 2022

This report supersedes any issued prior to 11 November 2022.

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 by quantitative nuclear magnetic resonance (qNMR). A combination of the six proton multiplets at 6.5-7.1 ppm and the one-proton triplet at 4.5 ppm were measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis, ^{1}H NMR spectroscopy, headspace GC-MS analysis of occluded solvent and elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler (2017 and 2018) or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler (2020) or Thermo Scientific Ultimate 3000 RS Tertiary pump, RS autosampler (2022).
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Methanol 0-10 min 15% B; 10-18 min 15-80% B; 18-28 min 80%B; 28-30 min 80-15%B; 30-40 min 15% B. The aqueous phase was buffered at pH 4.2 using 20mM NH_4OAc and AcOH.(2017, 2018 and 2020) or 20mM NH_4HCO_2 and HCOOH (2022).
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA or Shimadzu SPD-M20A PDA or RS RDA operating at 282 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.01% (10 sub samples in duplicate, September 2017)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 sub samples in duplicate, October 2018)
	Re-analysis:	Mean = 99.5%, s = 0.04% (5 sub samples in duplicate, February 2020)
	Re-analysis:	Mean = 99.3%, s = 0.03% (7 sub samples in duplicate, November 2022)
Karl Fischer analysis:		Moisture content 0.3% mass fraction (August 2017) Moisture content 0.4% mass fraction (September 2018) Moisture content 0.4% mass fraction (January 2020) Moisture content 0.4% mass fraction (September 2022)
Thermogravimetric analysis:		The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis. Non volatile residue 0.2% mass fraction (August 2017)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.5 ppm)
	Internal standard:	Dimethyl terephthalate (100% mass fraction)
	Initial analysis:	Mean (6.5-7.1 ppm) = 97.3%, s = 0.17% (4 sub samples, September 2017)
	Initial analysis:	Mean (4.6 ppm) = 97.6%, s = 0.05% (4 sub samples, September 2017)

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 0-10 min 15% B; 10-20 min 15-80% B; 20-30 min 80%B; 30-32 min 80-15%B. The aqueous phase was buffered at pH 3.0 using 10mM NH_4HCO_2 and AcOH
	Flow rate:	0.2 mL/min
	Sample prep:	2000 μg/g in mobile phase)
	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:	0.05 L/hr
	Desolvation gas flow:	600 L/hr
		The retention time of higenamine hydrochloride is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.
	4.78 min:	272.1 ($M+H^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Diethyl ether, ethanol, bromoethane
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (5:3) Single spot observed, R_f = 0.3
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3389, 3225, 2955, 2786, 1592, 1513, 1447, 1411, 1401, 1354, 1295, 1241, 1226, 1197, 1157, 1117, 886, 866, 837, 802, 784, 674, 522, 501 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 2.88-3.01 (3H, m), 3.27 (1H, quintet, *J* = 6.5 Hz), 3.40 (1H, dd, *J* = 5.6, 14.6 Hz), 3.48 (1H, quintet, *J* = 6.5 Hz), 4.63 (1H, dd, *J* = 5.7, 9.1 Hz), 6.69 (1H, s), 6.75 (1H, s), 6.88 (2H, d, *J* = 8.5 Hz), 7.17 (2H, d, *J* = 8.5 Hz) ppm
		Diethyl ether 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:	D_2O
	Spectral data:	δ 24.0, 38.3, 39.2, 56.2, 113.8, 115.7, 115.9, 123.3, 123.9, 126.7, 130.9, 142.8, 144.0, 154.9 ppm
Melting point:		280-285 °C
Microanalysis:	Found:	C = 60.7%; H = 5.8%; N = 4.7%; Cl = 11.4% (October 2017)
	Calculated:	C = 62.4%; H = 5.9%; N = 4.6%; Cl = 11.5% (Calculated for $C_{16}H_{17}NO_3$.HCl)