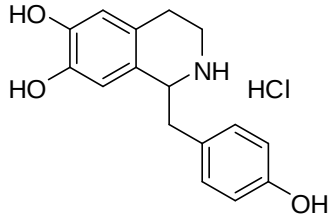
**REFERENCE MATERIAL PRODUCT INFORMATION SHEET**

**Report ID: D1070.2018.01**

Compound Name: **Higenamine hydrochloride**
Collection Number: D1070
Chemical Formula: $C_{16}H_{17}NO_3.HCl$
CAS Number: 11041-94-4
Structure:




Description: Off white solid
Batch Number: 17-D-04
Molecular Weight: 307.8 (HCl), 271.3 (base)
Release Date: 13th November 2017

Synonyms: 1,2,3,4-Tetrahydro-1-[(4-hydroxyphenyl)methyl]- 6,7-isoquinolinediol
(±)-Demethylcoclaurine hydrochloride

Purity (mass fraction): 97.3 ± 1.5% (95% coverage interval)

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.

| | | |
|---|---|---|
| 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) |
| HPLC: | Instrument: | Waters Model 1525 Binary pump, 717 plus autosampler |
| | Column: | X-bridge C-18, 5 µm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | A = MilliQ water; B = Methanol<br>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 |
| | Flow rate: | 1 mL/min |
| | Detector: | Waters PDA 2998 operating at 282 nm |
| | Relative peak area of 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) |
| 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) |
| Karl Fischer analysis: | | Moisture content 0.3% mass fraction (August 2017)<br>Moisture content 0.4% mass fraction (September 2018) |

**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<br>0-10 min 15% B; 10-20 min 15-80% B; 20-30 min 80%B; 30-32 min 80-15%B.<br>The aqueous phase was buffered at pH 4.2 using 10mM $NH_4OAc$ 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 temperature: 350 ºC |
| | Cone gas flow rate: | 0.05 L/hr | Desolvation gas flow rate: 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 | |
| IR: | Instrument: | Bruker Alpha FT-IR | |
| | 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<br>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)<br>Calc: C = 62.4%; H = 5.9%; N = 4.6%; Cl = 11.5%<br>(Calculated for $C_{16}H_{17}NO_3$.HCl) | |

**Expiration of certification**
The property values are valid till 17th October 2021, 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 long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of three years from the date of re-certification. 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.

In the absence of 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.

**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.

**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.

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

**Caution**
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

**Legal notice**
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R Davies
Team Leader,
Chemical Reference Materials, NMI
Dated: 27 November 2018

Characterisation data and property values specified in this report supersede all reports issued prior to 27th November 2018.