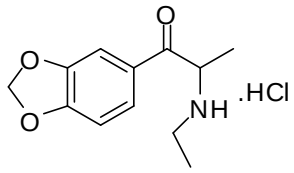
CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

Report ID: D959.2016.01

Compound Name: **(±)-*N*-Ethyl-3,4-methylenedioxycathinone hydrochloride**
Description: white crystalline solid
Collection Number: D959 Batch Number: 10-D-12
Chemical Formula: $C_{12}H_{16}ClNO_3$ Molecular Weight: 257.7
CAS Number: 1112937-64-0 (free base) Release Date: 11th January 2011
Structure:



Synonyms: 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)-1-propanone hydrochloride

Purity (mass fraction): 99.3 ± 1.9% (95% coverage interval)

The purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value by qNMR was obtained using the one-proton quartet at 5.05 ppm measured against a certified internal standard of potassium hydrogen maleate. Supporting evidence is provided by HPLC with UV detection, headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	120 °C (1 min), 10 °C/min to 200 °C, 30 °C/min to 300 °C (5 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component as the free base:		
	Initial analysis:	Mean = 99.8%, s = 0.04% (5 sub samples in duplicate, October 2013)	
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, August 2016)	
HPLC:	Instrument:	Shimadzu Model LC-20AB Binary pump, SIL-20A HT autosampler	
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)	
	Column oven:	40 °C	
	Mobile Phase:	Acetonitrile/MilliQ water (14:86 v/v)	
		Both aqueous and organic phases contained 0.05 % trifluoroacetic acid (v/v)	
	Flow rate:	1.0 mL/min	
	Detector:	Shimadzu PDA SPD-M20A operating at 282 nm	
	Relative peak area of main component:		
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, November 2010)	
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, October 2011)	
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, November 2012)	

Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (November 2010). The volatile content (e.g. organic solvents and/or water) could not be analysed accurately because of the inherent volatility of the material.
Karl Fischer analysis:		Moisture content ≤ 0.3% mass fraction (November 2010, 2011, 2012, October 2013 and August 2016)
QNMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	600 MHz Solvent: D_2O (4.71 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis:	Mean (5.05 ppm) = 99.3%, s = 0.64% (5 sub samples, November 2010)

Spectroscopic and other characterisation data

GC-MS:	Free base:		
	Instrument:	Agilent 6890/5973	
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 µm	
	Program:	60 °C (1 min), 10 °C/min to 100 °C, 15 °C/min to 250 °C (5 min), 30 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Transfer line temp: 300 °C
	Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1
	N-Acetyl derivative:		
	Instrument:	Agilent 6890/5973	
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 µm	
	Program:	60 °C (1 min), 10 °C/min to 100 °C, 15 °C/min to 250 °C (5 min), 30 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Transfer line temp: 300 °C
	Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1
	The retention times of the free base and *N*-acetyl derivative are reported along with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.		
	Free base (12.8 min): 219 (2), 149 (11), 121 (7), 91 (4), 72 (100), 70 (21), 65 (7), 44 (18), 42 (17) m/z *N*-acetyl (15.0 min): 263 (1), 178 (3), 149 (13), 121 (6), 114 (49), 72 (100), 65 (7), 44 (33) *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:	Ethanol and diethyl ether	
TLC:	Conditions:	Kieselgel $60F_{254}$. Methanol/NH_3 (100/1.5) Single spot observed, R_f = 0.68. Visualisation with UV at 254 nm	
IR:	Instrument:	Biorad FTS300MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	2970, 2793, 2458, 1675, 1605, 1555, 1453, 1258, 1120, 1089, 1039, 934, 871, 799, 753, 714, 520, 451 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III-400	
	Field strength:	400 MHz	Solvent: D_2O (4.79 ppm)
	Spectral data:	δ 1.36 (3H, t, *J* = 7.3 Hz), 1.60 (3H, d, *J* = 7.2 Hz), 3.12 (1H, qd, *J* = 7.3, 12.5 Hz), 3.21 (1H, qd, *J* = 7.3, 12.5 Hz), 5.05 (1H, q, *J* = 7.2 Hz), 6.11 (1H, d, *J* = 2.6 Hz), 6.12 (1H, d, *J* = 2.6 Hz), 7.01 (1H, d, *J* = 8.3 Hz), 7.44 (1H, d, *J* = 1.8 Hz), 7.67 (1H, dd, *J* = 1.8, 8.3 Hz) ppm	
		Ethanol estimated at 0.6% mass fraction was observed in the ^{1}H NMR. Diethyl ether was not detected.	
^{13}C NMR:	Instrument:	Bruker Avance III-400	
	Field strength:	100 MHz	Solvent: D_2O
	Spectral data:	δ 10.7, 16.0, 41.4, 57.7, 102.6, 107.9, 108.7, 126.5, 126.7, 148.3, 153.5, 195.3 ppm	
Melting point:		226-227 °C	
Microanalysis:		Found: C = 55.9%; H = 6.4%; N = 5.4%; Cl = 13.7% (November, 2010) Calc: C = 55.9%; H = 6.3%; N = 5.4%; Cl = 13.8% (Calculated for $C_{12}H_{16}ClNO_3$)	

Expiration of certification
The property values are valid till 30th August 2019, 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 demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

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.

Metrological traceability
The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis. 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).

Recommended storage
When not in use this material should be stored at or below 25 °C in a closed container protected from ambient moisture and light.

Intended use
For ***in vitro*** laboratory 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: 1 September, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 1st September, 2016.