Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

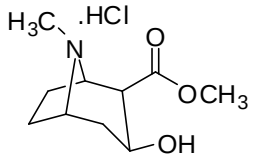
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D452c: Methyl ecgonine hydrochloride

Report ID: D452c.2023.01 (Bottled 160414)

Chemical Formula: $C_{10}H_{17}NO_3.HCl$

Molecular Weight: 235.7 g/mol (HCl), 199.3 g/mol (base)



Certified value

Batch No.	CAS No.	Purity (mass fraction)
12-D-22	38969-40-3 (HCl) 7143-09-1 (base)	92.9 ± 1.6%

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

IUPAC name: Methyl (1R,2R,3S,5S)-3-hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate hydrochloride.

Expiration of certification: The property values are valid till 10 October 2028, 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 prepared by synthesis, 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 GC-FID 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.
17 October 2023

This report supersedes any issued prior to 17 October 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:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include GC-FID/HPLC with UV/ELS detection thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

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

The certified purity value by qNMR was obtained using a combination of the six-proton multiplet at 2.0-2.5 ppm, the three-proton singlet at 2.8 ppm and the six-proton multiplet at 3.7-4.4 ppm measured against a certified internal standard of maleic acid.

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

GC-FID:	Instrument:	Varian-CP3800 or Agilent 6890N
	Column:	HP-1 or TG-17MS, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	100 °C (1 min), 10 °C/min to 160 °C (5 min), 30 °C/min to 300 °C (3 min)
	Injector:	200 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the free base:	
	Initial analysis:	Mean = 99.8%, s = 0.03% (10 sub samples in duplicate, February 2013)
	Re-analysis:	Mean = 99.8%, s = 0.06% (5 sub samples in duplicate, January 2014)
	Re-analysis:	Mean = 99.7%, s = 0.11% (5 sub samples in duplicate, January 2017)
	Re-analysis:	Mean = 99.8%, s = 0.10% (5 sub samples in duplicate, January 2020)
	Re-analysis:	Mean = 99.9%, s = 0.00% (5 sub samples in duplicate, October 2023)
Karl Fischer analysis:		Moisture content ca.7% mass fraction (February 2013, January 2014, November 2016 and November 2019)
		Moisture content 7.2% mass fraction (October 2023)
Thermogravimetric analysis:		The non-volatile residue < 0.1% mass fraction (February 2013). The volatile content (e.g. organic solvents and/or water) could not be determined because of the inherent volatility of the material and/or degradation at elevated temperatures.
QNMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	D_2O (4.79 ppm)
	Internal standard:	Maleic acid (98.7% mass fraction)
	Initial analysis:	Mean (2.0-2.5 ppm) = 93.2%, s = 0.1% (5 sub samples, February 2013)
	Initial analysis:	Mean (2.8 ppm) = 93.4%, s = 0.4% (5 sub samples, February 2013)
	Initial analysis:	Mean (3.7-4.4 ppm) = 93.4%, s = 0.7% (5 sub samples, February 2013)

Spectroscopic and other characterisation data

GC-MS:	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 300 °C (3 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the free base and *TMS* derivative are reported along with the major peaks in the mass spectrum. The latter are reported as *mass/charge* ratios and (in brackets) as a percentage relative to the base peak.	
	Free base (11.9 min):	199 (M^+, 14), 182 (8), 168 (12) 112 (10), 96 (70), 82 (100), 68 (10), 55 (12) *m/z*
	Mono-TMS (13.2 min):	271 (M^+, 8), 240 (8), 182 (13), 155 (11), 96 (75), 82 (100) *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
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol/aqueous conc. NH_3 (98.5/1.5) Single spot observed, R_f = 0.5
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3269, 2963, 2132, 1704, 1481, 1428, 1350, 1215, 1140, 1049, 1013, 968, 777, 616 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance 600
	Field strength:	600 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 2.04 - 2.15 (3H, m), 2.23 (1H, m), 2.31 - 2.38 (1H, m), 2.40 - 2.47 (1H, m), 2.84 (3H, s), 3.32 (1H, dd, *J* = 1.9, 7.1 Hz), 3.82 (3H, s), 4.00 (1H, m), 4.16 (1H, bd, *J* = 7.3 Hz), 4.46 (1H, dt, *J* = 12.5, 6.7 Hz) ppm. Diethyl ether (0.09%) and methanol (0.14%) estimated mass fraction was observed in the ^{1}H NMR in D_2O
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	D_2O
	Spectral data:	δ 22.5, 23.5, 34.8, 38.4, 48.8, 52.8, 60.3, 63.1, 63.8, 174.2 ppm
Melting point:		216-217 °C
Microanalysis:	Found:	C = 47.4%, H = 8.2%; N = 5.5%; Cl = 13.9% (February 2013)
	Calculated:	C = 47.3%, H = 8.0%; N = 5.5%; Cl = 14.0% (for $C_{10}H_{18}ClNO_3.H_2O$)
	Calculated:	C = 51.0%, H = 7.7%; N = 5.9%; Cl = 15.0% (for $C_{10}H_{18}ClNO_3$)