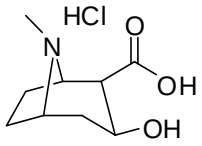
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D746b: Ecgonine hydrochloride



Report ID: D746b.2024.01

Chemical Formula: $C_9H_{15}NO_3.HCl$

Molecular Weight: 221.7 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
08-D-09	5796-31-6	99.3 ± 0.7%

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

IUPAC name: (1*R*,2*R*,3*S*,5*S*)-2-Carboxy-3-hydroxy-8-methyl-8-azoniabicyclo[3.2.1]octane chloride.

Expiration of certification: The property values are valid till 24 April 2029, 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: White powder prepared by synthesis, 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. 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 ten years. The measurement uncertainty at the 95% coverage 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 qNMR on five randomly selected 10-20 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 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.
27 June 2024

This report supersedes any issued prior to 22 June 2024.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The three-proton singlet at 2.8 ppm was measured against a certified internal standard of maleic acid.

Supporting evidence is provided by Karl Fischer analysis, ^{1}H NMR analysis and elemental microanalysis.

QNMR:	Instrument:	Bruker DMX
	Field strength:	400 and 600 MHz
	Solvent:	D_2O
	Internal standard:	Potassium hydrogen maleate
	Initial analysis:	Purity = 99.3% (mass fraction, n = 4, s = 0.2%, August 2008)
	Re-analysis:	Purity = 99.5% (mass fraction, n = 5, s = 0.8%, September 2009)
QNMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	D_2O
	Internal standard:	Maleic acid
	Initial analysis:	Purity = 99.1% (mass fraction, five samples in duplicate, s = 0.3%, January 2011)
	Re-analysis:	Purity = 99.1% (mass fraction, five samples in duplicate, s = 0.3%, November 2013)
QNMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	D_2O
	Internal standard:	Maleic acid
	Initial analysis:	Purity = 99.7% (mass fraction, five samples in duplicate, s = 0.2%, October 2016)
Karl Fischer analysis:		Moisture content 0.3% mass fraction (September 2008) Moisture content 0.2% mass fraction (January 2011 and November 2013) Moisture content $\leq$ 0.1% mass fraction (November 2016, September 2019 and April 2024)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	DB-1701, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 4 °C/min to 200 °C, 6 °C/min to 275 °C (6.5 min)
	Injector:	230 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.3 mL/min
	Split ratio:	Splitless
	The retention time of the *bis*-trimethylsilyl derivative is 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.	
	4.3 min:	329 (M^+, 9), 314 (15), 212 (11), 147 (12), 96 (62), 83 (100), 73 (39) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol/conc. NH_3 (200/3) Single spot observed, R_f = 0.27. Visualisation with ninhydrin.
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3267, 3091, 2964, 1709, 1431, 1351, 1227, 1140, 1041, 881, 607 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz
	Solvent:	D_2O (4.79)
	Spectral data:	δ 1.98-2.19 (4H, m), 2.25-2.41 (2H, m), 2.78 (3H, s), 3.18 (1H, dd, *J* = 2.3, 7.1 Hz), 3.92 (1H, m), 4.10 (1H, d, *J* = 7.3 Hz), 4.41 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	150 MHz
	Solvent:	D_2O
	Spectral data:	δ 23.2, 24.0, 35.5, 38.9, 49.5, 60.8, 63.6, 64.6, 176.4 ppm
Melting point:		233 °C
Microanalysis:	Found:	C = 49.1 %; H = 7.4 %; N = 6.3 % (August 2008)
	Calculated:	C = 48.8 %; H = 7.3 %; N = 6.3 % (Calculated for $C_9H_{15}NO_3$.HCl)