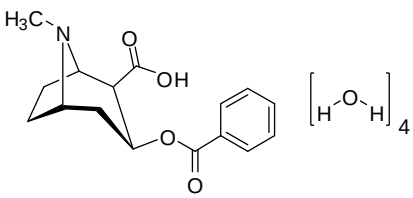
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D745c: Benzoylecgonine tetrahydrate



Report ID: D745c.2021.02 (Bottled 171113)

Chemical Formula: $C_{16}H_{19}NO_4.4H_2O$ (free base tetrahydrate), $C_{16}H_{19}NO_4$ (free base)

Molecular Weight: 361.4 (free base tetrahydrate), 289.3 (free base)

Certified value

Batch No.	CAS No.	Purity (mass fraction)
17-D-06	**5928-96-1 (free base tetrahydrate) 519-09-5 (free base)**	**99.5 ± 1.6%**

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*)-3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid

Expiration of certification: The property values are valid till 8 June 2024, 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. This material has been given a shelf life of three years from the date of re-certification.

Description: Off-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. 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 three 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 seven 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 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.
15 September 2022

This report supersedes any issued prior to 15 September 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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV 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.

Supporting evidence is provided by qNMR obtained using the one-proton doublet of doublets at 3.23 ppm measured against a certified internal standard of maleic acid, and elemental microanalysis.

Note: The purity value stated on this certificate represents the mass fraction of benzoyl ecgonine tetrahydrate in this material. The sole impurity is benzoic acid at 0.5% mass fraction. This material contains 20% water in line with literature precedents that benzoyl ecgonine exists as the tetrahydrate. The mass fraction of benzoyl ecgonine free base in this material is 79.6 ± 1.6% (95% coverage interval).

HPLC:	Instrument:	Waters Alliance 2695 or 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 (0.05% formic acid), B: Acetonitrile (0.05% formic acid)
	Flow rate:	1.0 mL/min
	Gradient:	0-10 min, 10%-50% B; 10-13 min, 50% B; 13-14 min, 50%-90% B; 14-15 min, 90%-10% B; 15-18 min, 10% B
	Detector:	Waters PDA 2998 operating at 232 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.2% (7 sub samples in duplicate, August 2017)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, July 2018)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, June 2021)
Thermogravimetric analysis:		Volatile content 20.1% and non volatile residue < 0.1% mass fraction (August 2017)
Karl Fischer analysis:		Moisture content 20.0% mass fraction (August 2017) Moisture content 20.0% mass fraction (September 2018) Moisture content 18.8% mass fraction (May 2021)
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 (3.23 ppm) = 80.4%, s = 0.2% (5 sub samples, August 2017)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	HP-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

Mono-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	60 °C (1 min), 10 °C/min to 250 °C, 30 °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 parent compound and *mono*-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.

Parent (22.3 min):	289 (M^+, 8), 168 (48), 124 (100), 105 (34), 94 (24), 82 (32), 77 (38) *m/z*
Mono-TMS (20.2 min):	361 (M^+, 17), 309 (46), 240 (62), 179 (35), 105 (65), 94 (31), 82 (100), 75 (47) *m/z*

IR:

Instrument:	BioRad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3504, 3416, 1720, 1601, 1451, 1351, 1275, 1116, 716 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance 500
Field strength:	500 MHz
Solvent:	D_2O (4.79 ppm)
Spectral data:	δ 2.07-2.20 (2H, m), 2.29-2.50 (4H, m), 2.80 (3H, s), 3.15 (1H, dd, *J* = 2.6, 7.0 Hz), 3.98 (1H, d, *J* = 5.0 Hz), 4.04 (1H, m), 5.49 (1H, m), 7.51 (2H, t, *J* = 7.8 Hz), 7.66 (1H, t, *J* = 7.5 Hz), 7.98 (2H, dd, *J* = 1.4, 7.2 Hz) ppm.
	Benzoic acid estimated at 0.5% mass fraction was observed in the ^{1}H NMR

^{13}C NMR:

Instrument:	Bruker DMX-300
Field strength:	75.5 MHz
Solvent:	D_2O
Spectral data:	δ 23.2, 32.6, 37.6, 48.8, 62.3, 64.8, 64.8, 128.7, 128.9, 129.5, 133.9, 167.2, 176.9 ppm

Melting point: 91-92 °C

Microanalysis:

Found:	C = 53.4 %; H = 7.5 %; N = 3.9 % (September 2017)
Calculated:	C = 53.2 %; H = 7.5 %; N = 3.9 % (for $C_{16}H_{19}NO_4.4H_2O$)