Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

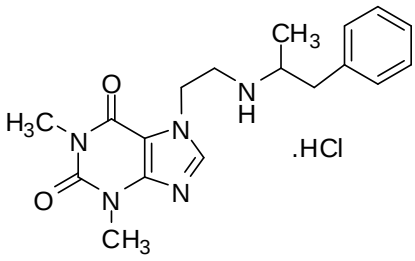
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D543b: Fenethylline hydrochloride

Report ID: D543b.2024.01

Chemical Formula: $C_{18}H_{23}N_5O_2$.HCl

Molecular Weight: 377.9 g/mol (HCl), 341.4 g/mol (base)



Certified value

Batch No.	CAS No.	Purity (mass fraction)
08-D-03	1892-80-4 (HCl) 3736-08-1 (base)	98.7 ± 1.3%

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

IUPAC name: 1,3-Dimethyl-7-{2-[(1-phenyl-2-propanyl)amino]ethyl}-3,7-dihydro-1H-purine-2,6-dione hydrochloride

Expiration of certification: The property values are valid till 12 September 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: Crystalline white solid prepared by synthesis, certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium 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%.

Stability: This material has demonstrated stability over a minimum period of five 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 GC-FID on five randomly selected 1-2 mg sub samples of the material. The material was judged to be 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.
20 September 2024.

This report supersedes any issued prior to 20 September 2024.

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 GC-FID, 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 elemental microanalysis.

GC-FID:	Instrument:	Varian 3800 or Agilent 7890
	Column:	HP-1 29.95m x 0.32mm x 0.25 μm
	Program:	120 °C (1 min), 20 °C/min to 250 °C (7 min), 30 °C/min to 300 °C (3min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.02% (7 sub samples in duplicate, March 2008)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, March 2009)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 sub samples in duplicate, March 2010)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 sub samples in duplicate, February 2011)
	Re-analysis	Mean = 99.6%, s = 0.02% (5 sub samples in duplicate, January 2016)
	Re-analysis	Mean = 99.4%, s = 0.06% (5 sub samples in duplicate, October 2020)
	Re-analysis	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, September 2024)
Thermogravimetric analysis:		Volatile and non-volatile content not determined by this technique.
Karl Fischer analysis:		Moisture content 0.3% mass fraction (March 2009) Moisture content ≤ 0.2% mass fraction (March 2010, December 2015, November 2020 and September 2024)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	ZB-5ms, 28 m × 0.25 mm × 0.25 μm
	Program:	50 °C (1 min), 30 °C/min to 280 °C, (5 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention time of the fenethylline free base 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.	
	11.1 min:	251 (15), 250 (100), 208 (4), 207 (35), 181 (4), 148 (4), 119 (4), 91 (16), 70 (10), 56 (3) *m/z*
ESI -MS:	Instrument:	Waters Acquity UPLC/MS
	Operation:	Positive ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	3.47 kV
	Cone voltage:	6 V
	Peak:	342.2 (M+H) + *m/z*
TLC:	Conditions:	Kieselgel 60F254. Methanol / Chloroform (1/9) Single spot observed, Rf = 0.3. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	2945, 2716, 2423, 1699, 1660, 1543, 1459, 1236, 1190, 1029, 979, 745 cm^{-1}
^{1}H NMR:	Instrument:	DMX500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 1.27 (3H, d, *J* = 6.5 Hz), 2.76 (1H, dd, *J* = 9.8, 13.2 Hz), 3.24 (1H, dd, *J* = 4.7, 13.3 Hz), 3.36 (3H, s), 3.56 (3H, s), 3.57-3.73 (3H, m), 4.71 (2H, t, *J* = 6.3 Hz), 7.26-7.35 (5H, m), 8.06 (1H, s) ppm
		Ethanol < 0.5% mass fraction was observed in the ^{1}H NMR.
^{13}C NMR:	Instrument:	DPX-300
	Field strength:	75 MHz
	Solvent:	MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 15.9, 28.4, 30.2, 40.1, 44.4, 46.1, 57.6, 108.3, 128.4, 130.0, 130.4, 137.0, 144.4, 150.6, 153.0, 157.0 ppm
Melting point:		225-227 °C
Microanalysis:	Found:	C = 57.2 %; H = 6.4 %; N = 18.7%
	Calculated:	C = 57.2 %; H = 6.4 %; N = 18.5% (Calculated for $C_{18}H_{23}N_5O_2$.HCl)