Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

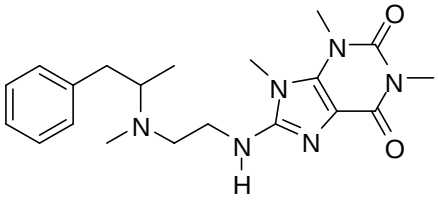
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D913: Fencamine

Report ID: D913.2019.01 (Bottled 150908)

Chemical Formula: $C_{20}H_{28}N_6O_2$

Molecular Weight: 384.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
06-D-10	28947-50-4	99.0 ± 0.3%

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

IUPAC name: 1,3,7-Trimethyl-8-({2-[methyl(1-phenyl-2-propanyl)amino]ethyl}amino)-3,7-dihydro-1H-purine-2,6-dione

Expiration of certification: The property values are valid till 13 November 2024, 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%.

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.
9 December 2019

This report supersedes any issued prior to 9 December 2019

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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, HPLC, 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 qualitative elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N/Agilent 7890A
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 μm
	Program:	220 °C (1 min), 20 °C/min to 300 °C (10 min) or 200 °C (1 min), 30 °C/min to 300 °C (10 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.04% (3 sub samples in duplicate, April 2007)
	Re-analysis:	Mean = 99.1%, s = 0.02% (5 sub samples in duplicate, April 2008)
	Re-analysis:	Mean = 99.1%, s = 0.02% (5 sub samples in duplicate, March 2012)
	Re-analysis:	Mean = 99.2%, s = 0.01% (5 sub samples in duplicate, February 2015)
	Re-analysis:	Mean = 99.2%, s = 0.04% (5 sub samples in duplicate, November 2019)
	Instrument:	Varian CP3800
	Column:	VF-1, 30 m x 0.32mm x 0.25um
	Program:	220 °C (1 min), 20 °C/min to 300 °C (10 min)
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s= 0.07% (5 sub samples in duplicate, April 2009)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Methanol/Potassium phosphate buffer pH 2.0 (6.6 g KH_2PO_4)/L 0-1 min 20% B; 1-5 min 20-60% B; 5-10 min 60%B; 10-11 min 40-20%B, 11-20 min 20%B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 200-235 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.7%, s = 0. 1% (10 sub samples in duplicate, November 2006)
Karl Fischer analysis:		Moisture content ≤ 0.3% mass fraction (October 2006, April 2008, March 2009, March 2012, February 2015 and November 2019)
Thermogravimetric analysis:		Initial volatile content < 0.1% and non-volatile residue < 0.2 % mass fraction. (October 2006) Re-analysis volatile content < 0.1% and non-volatile residue = 0.3 % mass fraction.(3 sub samples, April 2008) Re-analysis volatile content < 0.1% and non-volatile residue< 0.3 % mass fraction (April 2009)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	HP-2, 17 m × 0.20 mm I.D. × 0.25 µm
	Program:	87 °C (1.5 min), 20 °C/min to 160 °C, 35 °C to 310 °C (6 min)
	Injector:	260 °C
	Split ratio:	20/1
	Transfer line temp:	300 °C
	The retention time of the parent compound is reported 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.	
	10.7 min:	384 (M^+, 11), 293 (76), 236 (58), 209 (15), 176 (15), 162 (100), 146 (10), 119 (27), 91 (62), 84 (27), 58 (10), 42 (15) m/z
ESI-MS:	Instrument:	Micromass Quattro Micro
	Operation:	Positive ion mode, direct infusion at 5 µL/min
	Ionisation:	ESI spray voltage at 3.2 kV positive ion
	EM voltage:	650 V
	Cone voltage:	35 V
	Peak:	385 ($M+H^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol/concentrated aq.NH_3 (98.5/1.5) Single spot observed, R_f = 0.42. Visualisation with UV at 254nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3332, 2960, 1707, 1662, 1614, 1582, 1550, 1456, 1221, 1030, 974, 743, 700 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$
	Spectral data:	δ 1.04 (3H, d, *J* = 6.5 Hz), 2.26 (3H, s), 2.58-2.65 (2H, m), 2.68-2.72 (1H, m), 2.80 (1H, dd, *J* = 8.9, 14.1 Hz), 3.05-3.09 (1H, m), 3.21 (3H, s), 3.36 (3H, s), 3.37-3.40 (2H, m), 3.49 (3H, s), 4.66 (1H, bs), 7.16-7.2 (3H, m), 7.24-7.28 (2H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX-600
	Field strength:	151 MHz
	Solvent:	$CDCl_3$
	Spectral data:	δ 13.3, 27.5, 29.2, 29.6, 34.9, 39.8, 40.0, 52.0, 59.7, 103.0, 126.0, 128.4, 128.8, 140.4, 148.6, 151.7, 153.4, 154.1 ppm
Melting point:		152-153 °C
Microanalysis:	Found:	C = 62.5%; H = 7.4%; N = 22.0%
	Calculated:	C = 62.5%; H = 7.3%; N = 21.9% (Calculated for $C_{20}H_{28}N_6O_2$)