Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

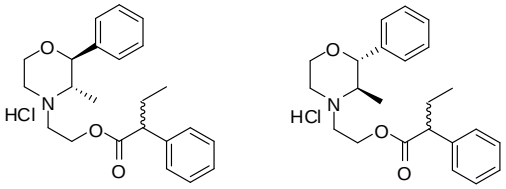
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D915: Fenbutrazate hydrochloride

Report ID: D915.2023.01 (Ampouled 111205)

Chemical Formula: $C_{23}H_{29}NO_3$.HCl

Molecular Weight: 403.9 g/mol (HCl), 367.5 g/mol (base)



Certified value

Batch No.	CAS No.	Mass per ampoule
06-D-16	6474-85-7	966 ± 14 μg

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

IUPAC name: 3-Methyl-2-phenyl-4-{2-[(2-phenylbutanoyl)oxy]ethyl}morpholin-4-ium chloride

Expiration of certification: The property values are valid till 11 April 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D915. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform/$NaHCO_3$). This will transfer 966 ± 14 μg of anhydrous fenbutrazate hydrochloride. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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: 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 seven randomly selected ampoules 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.
13 April 2023

This report supersedes any issued prior to 13 April 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:

GC-FID:	Instrument:	Agilent 6890 or 8890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	250 °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 = 99.4%, s = 0.01% (7 ampoules in duplicate, December 2011)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 ampoules in duplicate, December 2012)
	Re-analysis:	Mean = 99.2%, s = 0.06% (5 ampoules in duplicate, October 2015)
	Re-analysis:	Mean = 99.2%, s = 0.06% (5 ampoules in duplicate, September 2018)
	Re-analysis:	Mean = 99.2%, s = 0.06% (5 ampoules in duplicate, April 2023)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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.

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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 6890
	Column:	HB-1, 29.5 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	250 °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 = 99.5%, s = 0.01% (10 sub samples in duplicate, March 2007)
	Re-analysis:	Mean = 99.4%, s = 0.01% (5 sub samples in duplicate, February 2008)
	Re-analysis:	Mean = 99.4%, s = 0.01% (5 sub samples in duplicate, January 2009)
	Re-analysis:	Mean = 99.5%, s = 0.03% (5 sub samples in duplicate, January 2010)
Thermogravimetric analysis:		Volatile content <0.1% and non volatile residue < 0.2% mass fraction (February 2008)
Karl Fischer analysis:		Moisture content < 0.4% mass fraction. (April 2007, February 2008, January 2009 and January 2010)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	ZB-5 MS, 28 m × 0.25 mm I.D. × 0.25 μm
	Program:	60 °C (1 min), 15 °C/min to 300 °C, (5 min)
	Injector:	200 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention time of the 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.	
	Free base (16.2 min):	367 (M^+, 8), 261 (66), 246 (19), 233 (8), 190 (48), 119 (58), 114 (40), 98 (60), 91 (100), 84 (46), 69 (92), 56 (66), 42 (15) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (1/4) Single spot observed, R_f = 0.58. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	2964, 2935, 2874, 2426, 1734, 1454, 1164, 1117, 1007, 761, 701 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.30 ppm)
	Spectral data:	δ 0.87 (3H, t, *J* = 7.3 Hz), 1.03 (3H, t, *J* = 7.2 Hz), 1.82 (1H, m), 2.12 (1H, m), 3.11-3.25 (2H, m), 3.35-3.5 (2H, m), 3.61 (1H, m), 3.71 (1H, m), 3.92-4.03 (2H, m), 4.42-4.58 (3H, m), 7.20-7.44 (10H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 12.2, 13.0, 12.9, 27.1, 53.05, 53.12, 53.5, 53.6, 54.1, 59.6, 59.7, 64.2, 64.3, 64.9, 83.4, 128.5, 128.6, 129.0, 129.2, 129.3, 129.88, 129.92, 130.4, 138.5, 140.0, 174.5 ppm
Melting point:		137-142 °C
Microanalysis:	Found:	C = 68.7 %; H = 7.3 %; N = 3.4% (June 2007)
	Calculated:	C = 68.4 %; H = 7.5 %; N = 3.5% (Calculated for $C_{23}H_{29}NO_3$.HCl)

The Synthesis and Certification of this Reference Material is supported by the Australian Government through the *Anti-Doping Research Program (ADRP)* of the Department of Communications, Information Technology and the Arts.