Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

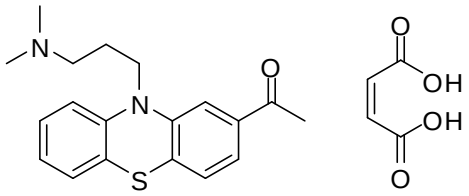
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA M259: Acepromazine maleate

Report ID: M259.2021.02

Chemical Formula: $C_{23}H_{26}NO_5S$

Molecular Weight: 442.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
06-AV-03	**3598-37-6**	**97.5 ± 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-[10-[3-(Dimethylamino)propyl]-10H-phenothiazin-2-yl]ethanone (2Z)-2-butenedioate.

Expiration of certification: The property values are valid till 14 October 2031, i.e. ten 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: Yellow crystals sourced from an external supplier, 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 5 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 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 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.
12 October 2022

This report supersedes any issued prior to 12 October 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 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 qualitative elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N Agilent 8890 (2021)		
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 µm		
	Program:	200 °C (1 min), 10 °C/min to 300 °C (5 min)		
	Injector:	250 °C	Detector Temp:	320 °C
	Carrier:	Helium	Split ratio:	20/1
	Relative mass fraction of the main componentas the free base:			
	Initial analysis:	Mean = 98.7%, s = 0.02% (7 sub samples in duplicate, May 2016)		
	Re-analysis:	Mean = 98.7%, s = 0.03% (5 sub samples in duplicate, March 2012)		
	Re-analysis:	Mean = 98.3%, s = 0.1% (5 sub samples in duplicate, March 2017)		
	Re-analysis:	Mean = 98.7%, s = 0.1% (5 sub samples in duplicate, October 2021)		
Karl Fischer analysis:		Moisture content 0.8% mass fraction (June 2008 & May 2009 and March 2017) Moisture content 0.4% mass fraction (April 2012) Moisture content 0.5% mass fraction (November 2021)		
Thermogravimetric analysis:		Volatile content not determined and non volatile residue < 0.2 % mass fraction (May 2006 & June 2007)		

Spectroscopic and other characterisation data

GC-MS:	Free base:	
	Instrument:	HP5890/5971A
	Column:	ZB-5ms, 26 m × 0.25 mm I.D. × 0.25 μm
	Program:	220 °C (2 min), 10 °C/min to 290 °C (5 min), 10 °C/min to 300 °C
	Injector:	250 °C
	Split ratio:	40/1
	Transfer line temp:	280 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention time of the free base 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.	
	Parent (8.78 min):	326 (M^+, 15), 280 (8), 241 (5), 197 (5), 196 (5), 86 (19), 85 (9), 58 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol / conc. ammonia (100/1.5) Single spot observed, R_f = 0.51
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3600-3400 broad, 3054, 3010, 2957, 2863, 2800-2200 broad, 1676, 1580, 1459, 1417, 1356, 1234, 1221, 874, 743, 732 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 2.23 (2H, tt, *J* = 6.2, 7.6 Hz), 2.55 (3H, s), 2.70 (6H, s), 3.13 (2H, t, *J* = 7.7 Hz), 4.07 (2H, t, *J* = 6.2 Hz), 6.19 (2H, s), 6.89 (1H, d, *J* = 8.1 Hz), 6.98 (1H, t, *J* = 7.4 Hz), 7.16 (1H, dd, *J* = 1.2, 7.6 Hz), 7.20 (1H, ddd, *J* = 1.4, 7.4, 7.4 Hz), 7.23 (1H, d, *J* = 8.0 Hz), 7.41 (1H, d, *J* = 1.4 Hz), 7.50 (1H, dd, *J* = 1.6, 8.0 Hz) ppm
	Ethyl acetate at 0.7% mass fraction was determined from the ^{1}H NMR (March 2006, June 2007 & June 2009)	
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	125 MHz
	Solvent:	$CDCl_3$ (77.16 ppm)
	Spectral data:	δ 21.6, 26.6, 42.8, 44.0, 55.3, 114.2, 116.1, 123.6, 123.7, 124.9, 127.5, 127.8, 128.0, 133.0, 135.4, 136.4, 143.6, 145.0, 169.4, 197.2 ppm.
Melting point:		133-135 °C
Microanalysis:	Found:	C = 62.2%; H = 6.1%; N = 6.4%; (May, 2006)
	Calculated:	C = 62.4%; H = 5.9%; N = 6.3%; (Calculated for $C_{23}H_{26}NO_5S$)