Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

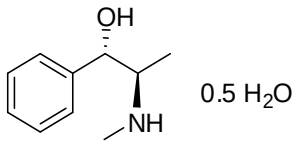
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1090: (+)-Ephedrine hemihydrate

Report ID: D1090.2024.01

Chemical Formula: $C_{10}H_{15}NO.\ ½\ H_2O$

Molecular Weight: 174.2 g/mol (hemihydrate), 165.2 (base)



Certified value

Batch No.	CAS No.	Purity (mass fraction of ephedrine free base)
24-D-01	**321-98-2 (free base)**	**93.8 ± 1.4%**

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

IUPAC name: (1S,2R)-2-(Methylamino)-1-phenyl-1-propanol hydrate.

Expiration of certification: The property values are valid till 24 January 2034, 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: Off white crystalline solid 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 4 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 all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of ten years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from long term 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.
26 February 2024
NATA Accreditation No. 198 / Corporate Site No. 14214.

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.

Note: This material is nominally the hemihydrate in which water of crystallisation constitutes 5.2% mass fraction. The certified purity value is stated as the mass fraction of ephedrine free base (93.8 ± 1.7% mass fraction) and the water content (5.3% mass fraction) is treated as an impurity.

GC-FID:	Instrument:	Varian CP-3800
	Column:	DB-17, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	160 °C (1 min), 5 °C/min to 190 °C (5 min), 30 °C/min to 280 °C (5 min)
	Injector:	180 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component as the *O*,*N*-*bis*-acetyl derivative:	
	Initial analysis:	Mean = 99.9%, s = 0.0% (10 sub samples in duplicate, October 2000)
	Re-analysis:	Mean = 99.4%, s = 0.1% (5 sub samples in duplicate, November 2010)
	Re-analysis:	Mean = 99.1%, s = 0.1% (7 sub samples in duplicate, January 2024)
HPLC:	Instrument:	Waters
	Column:	Waters X-Terra RP18, 5 µm (3.9 mm × 150 mm)
	Mobile Phase:	A = 5% Acetonitrile/95% H_2O containing 2% by volume conc. NH_4OH B = 100% Acetonitrile Gradient: 100% A hold 1 min, to 50% A over 15 min, hold 10 min
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 211 nm
	Relative mass fraction of the main component as the *O*,*N*-*bis*-acetyl derivative:	
	Initial analysis:	Mean = 99.8%, s = 0.4% (3 sub samples in duplicate November 2000)
Karl Fischer analysis:		Moisture content = 5.2 % mass fraction (November 2006) Moisture content = 5.3 % mass fraction (December 2023)
Thermogravimetric analysis:		Non-volatile residue < 0.2 % mass fraction (March 2001)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	HP6890/5973
	Column:	HP Ultra 2, 17 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)
	Injector:	260 °C
	Split ratio:	30/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	Pentafluorobenzyl derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 2, 17 m x 0.20 mm ID x 0.11 μm
	Program:	95 °C, 35 °C/min to 175 °C (6 min), then 35 °C/min to 310 °C (3 min)
	Injector:	260 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	The retention time of the ephedrine free base and *PFB* derivative are 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.	
	Ephedrine (3.72 min):	105 (3), 79 (4), 77 (10), 58 (100), 56 (4) *m/z*
	PFB derivative (7.3 min):	253 (34), 252 (56), 234 (13), 218 (10), 195 (100), 167 (12) *m/z*
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, KBr
	Peaks:	3300, 2981, 1490, 1453, 1349, 1160, 1086, 993, 749, 701 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	CDCl3 (7.23 ppm)
	Spectral data:	δ 0.85 (3H, d), 2.38 (3H, s), 2.72 (1H, m), 4.70 (1H, d), 7.29 (5H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz
	Solvent:	$CDCl_3$ (77 ppm)
	Spectral data:	δ 13.8, 33.7, 60.3, 73.4, 126.0, 126.9, 128.0, 141.8 ppm
Melting point:		40-41 °C
Microanalysis:	Found:	C = 69.2%; H = 9.2%; N = 8.0% (November 2000)
	Found:	C = 68.8%; H = 9.2%; N = 8.0% (May 2002)
	Found:	C = 69.2%; H = 9.2%; N = 8.1% (August 2005)
	Calculated:	C = 68.9%; H = 9.3%; N = 8.0% (Calculated for $C_{10}H_{15}NO.$ ½ H_2O)
Base titration:		Acid-base titration of ephedrine: 94.1% mass fraction, s = 0.45% (November 2000)