



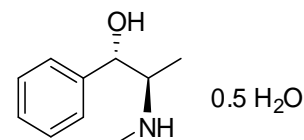
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA M723: (+)-Ephedrine hemihydrate

Report ID: M723.2021.02

Chemical Formula: C₁₀H₁₅NO. ½ H₂O

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



Certified value

Batch No.	CAS No.	Purity (mass fraction of Ephedrine base)
00-D-06	321-98-2 (free base)	87.5 ± 1.2%

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 30 July 2024, i.e. three 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: 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 25 °C 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 three 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 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.
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 ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by qualitative elemental microanalysis.

Note: This material is nominally the hemihydrate in which water of crystallisation constitutes 5.2% mass fraction. In this certified reference material the water of crystallisation has decreased over time and the main component can no longer be accurately described as a hemi hydrate. To address this deficiency of water the certified purity value is stated as the mass fraction of ephedrine free base (87.5 ± 1.2% mass fraction) and the water content (4.5% mass fraction) is treated as an impurity.

GC-FID:	Instrument: Varian CP-3800 Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 µm Program: 90 °C (1 min), 8 °C/min to 160 °C, 30 °C/min to 280 °C (5 min) Injector: 200 °C Detector Temp: 320 °C Carrier: Helium Split ratio: 20/1 Relative mass fraction of the main component: Initial analysis: Mean = 97.1%, s = 0.2% (7 sub samples in duplicate, August 2018) Re-analysis: Mean = 96.6%, s = 0.2% (5 sub samples in duplicate, July 2021)
GC-FID:	Instrument: HP5890 Column: SGE BPX-5 Capillary, 20 m × 0.32 mm I.D. × 0.25 µm Program: 90 °C (1 min), 5 °C/min to 150 °C, 40 °C/min to 275 °C (3 min) Injector: 250 °C Detector Temp: 325 °C Carrier: Helium Split ratio: 15/1 Relative mass fraction of the main component: Initial analysis: Mean > 99.9% (10 sub samples in duplicate, October 2000)
GC-FID:	Instrument: Varian CP-3800 Column: VF-1, 30 m × 0.32 mm I.D. × 0.25 µm Program: 90 °C (1 min), 10 °C/min to 150 °C (6 min), 20 °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: Initial analysis: Mean = 99.4%, s = 0.1% (5 sub samples in duplicate, November 2010) Re-analysis: Mean = 98.3%, s = 0.1% (5 sub samples in duplicate, September 2015)
HPLC:	Instrument: Waters Column: Waters X-Terra RP18, 5 µm (3.9 mm × 150 mm) Mobile Phase: A = 5% Acetonitrile/95% H ₂ O containing 2% by volume conc. NH ₄ OH 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 peak area of the main component: 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 = 5.1 % mass fraction (November 2010) Moisture content = 4.9 % mass fraction (September 2015) Moisture content = 4.7 % mass fraction (July 2018) Moisture content = 4.5 % mass fraction (August 2021)
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 μ m
 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 μ m
 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}

¹H 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

¹³C NMR: Instrument: Bruker DMX-500
 Field strength: 125 MHz
 Solvent: CDCl₃ (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 C₁₀H₁₅NO. ½ H₂O)

Base titration: Acid-base titration of ephedrine: 94.1% mass fraction, *s* = 0.45%