The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1) S (1) 1095-1102, 2026 www.thebioscan.com

Development and Validation of a Reverse Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Gabapentin and Mecobalamin in Tablet Dosage Form

M. Noor Basha, P.T. Nagaraju*, Dr. J. Gopala Krishna, Dr. B.V. Ramana

Department of Pharmaceutical Analysis, Dr. K.V. Subba Reddy Institute of Pharmacy, Kurnnol, A.P, India-518218.

P.T. Nagaraju
Assistant Professor,
Department of Pharmaceutical Analysis
E-mail: Nraju04@gmail.com

DOI: https://doi.org/10.63001/tbs.2026.v21.i01.S.I(1).pp1095-1102

KEYWORDS

Gabapentin,
Mecobalamin,
RP-HPLC,
Method validation,
ICH guidelines

Received on: 28-03-2026

Accepted on: 19-03-2026

Published on: 31-03-2026

Abstract

A simple, precise, accurate, and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Gabapentin and Methylcobalamin (Mecobalamin) in tablet dosage form. Chromatographic separation was achieved using an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of Methanol and phosphate buffer (60:40 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 271 nm. The retention times were found to be 2.955 min for Gabapentin and 3.532 min for Mecobalamin. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, precision, accuracy, ruggedness, and robustness. Linearity was observed in the concentration range of 20–80 ppm for both drugs with correlation coefficient (r^2) of 0.999. The % recovery was within 98–102%, and %RSD values were below 2%. The developed method was found suitable for routine quality control analysis of combined dosage forms.

INTRODUCTION

Pharmaceutical analysis plays a vital role in quality assurance and quality control of bulk drugs and pharmaceutical formulations. It involves qualitative and quantitative determination of chemical components using advanced analytical techniques. High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical industries due to its accuracy, reproducibility, and sensitivity (Snyder et al., 1997). Dong (2006) described HPLC as a highly efficient separation technique capable of analyzing complex

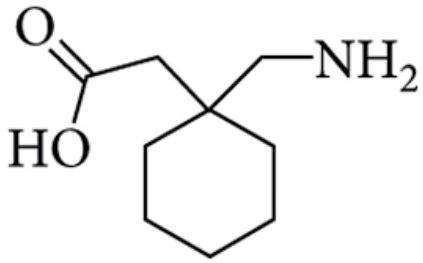
mixtures with high resolution. Reversed phase chromatography, particularly using C18 bonded silica columns, is most commonly employed for analysis of polar and semi-polar pharmaceutical compounds.

Gabapentin is an anticonvulsant and neuropathic pain agent structurally related to gamma-aminobutyric acid (GABA), although it does not directly interact with GABA receptors. It is widely used in the management of epilepsy and peripheral neuropathy.

Methylcobalamin (Mecobalamin) is an active form of Vitamin B12 that plays an essential role in neurological function and red blood cell formation. It is commonly prescribed in combination with Gabapentin for the treatment of neuropathic disorders. Several analytical methods have been reported for estimation of Gabapentin and Mecobalamin individually or in combination, including UV spectrophotometric and HPLC methods (Baheti and Galande, 2011; Goti and Patel, 2013). However, there remains a need for a simple, economical, and validated RP-HPLC method suitable for routine quality control. Therefore, the present study aimed to develop and validate a robust RP-HPLC method for simultaneous estimation of Gabapentin and Mecobalamin in tablet dosage form.

Drug Profile

Gabapentin

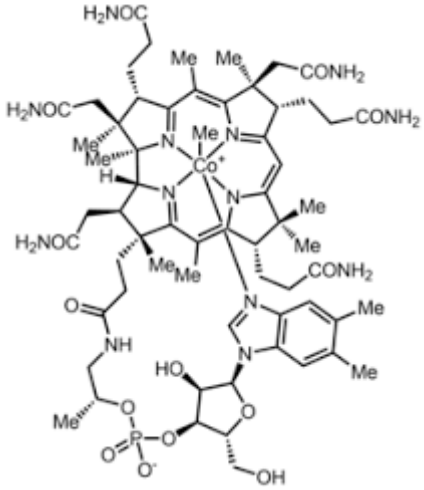


Chemical Name: 1-(Aminomethyl) cyclohexane acetic acid
Molecular Formula: C9H17NO2
Molecular Weight: 171.24 g/mol
Category: Anticonvulsant, Neuropathic analgesic

Pharmacological Action

Gabapentin binds to the α2δ subunit of voltage-gated calcium channels in the central nervous system, reducing excitatory neurotransmitter release. It is widely used in epilepsy, post-herpetic neuralgia, and diabetic neuropathy.

Mecobalamin (Methylcobalamin)



Chemical Name: Methylcobalamin
Molecular Formula: C63H91CoN13O14P
Category: Vitamin B12 analogue
Pharmacological Action

Mecobalamin acts as a cofactor in methionine synthesis and nucleic acid metabolism. It promotes myelin sheath formation and nerve regeneration, making it beneficial in peripheral neuropathy and megaloblastic anemia.

MATERIALS AND METHODS

Instruments

- HPLC system – Waters Model 2690/5 series
- Column – Inertsil ODS C18 (250 × 4.6 mm, 5 µm)
- Electronic balance – Sartorius
- Sonicator – Fast Clean

Chemicals and Reagents

- Methanol (HPLC grade)
- Potassium dihydrogen phosphate (Buffer)
- Gabapentin working standard
- Mecobalamin working standard

Optimized Chromatographic Conditions

- Mobile Phase: Methanol : Buffer (60:40 v/v)
- Flow rate: 1.0 mL/min
- Detection wavelength: 271 nm
- Injection volume: 20 µL
- Column temperature: Ambient
- Retention times: 2.955 min (Gabapentin), 3.532 min (Mecobalamin)

Method Validation (ICH Q2 R1)

- **System suitability:** %RSD < 2%, theoretical plates > 3000
- **Specificity:** No interference from excipients
- **Linearity:** 20–80 ppm ($r^2 = 0.999$)
- **Accuracy:** 98–102% recovery
- **Precision:** %RSD < 2%

- **Ruggedness & Robustness:** Passed systems under varied flow rates and different

RESULTS & DISCUSSION

System Suitability

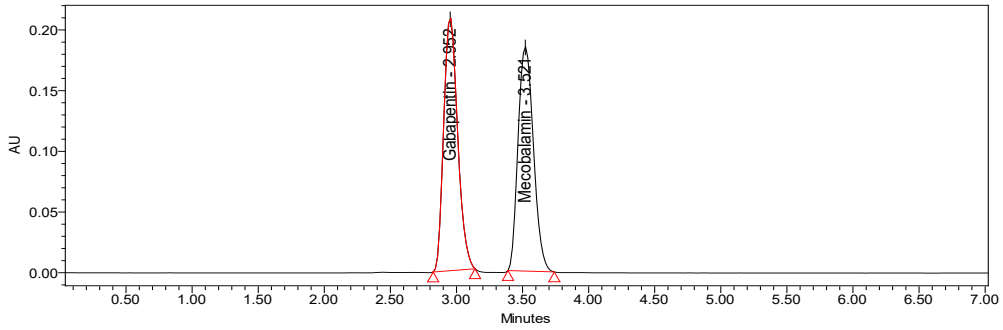


Fig: 1: Chromatogram of system suitability

SPECIFICITY

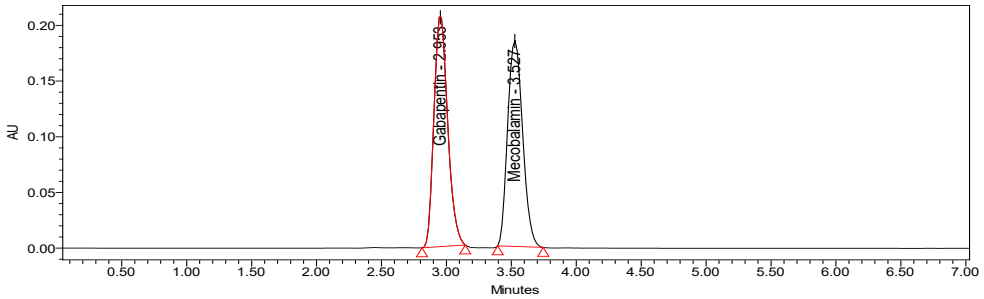


Fig 2: Chromatogram of sample

PRECISION

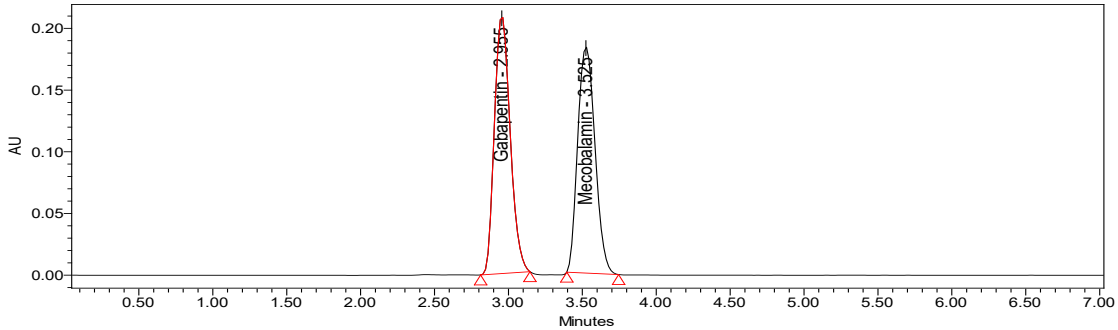
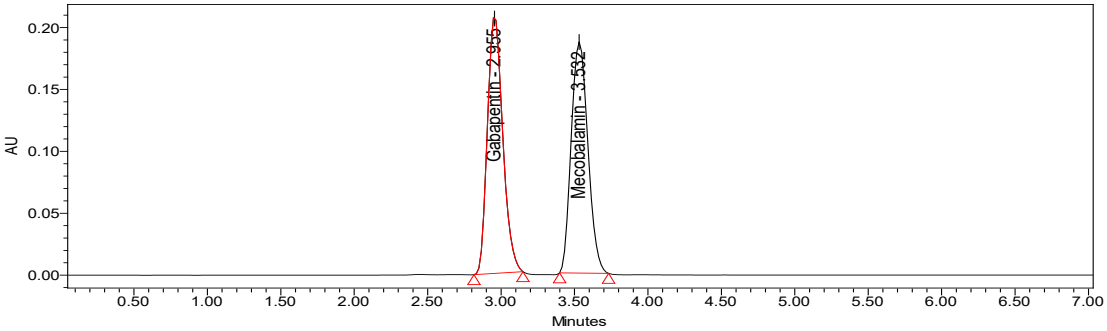


Fig-3: Chromatograms of system precision



Fig– 4: Chromatograms of Intermediate precision

ACCURACY

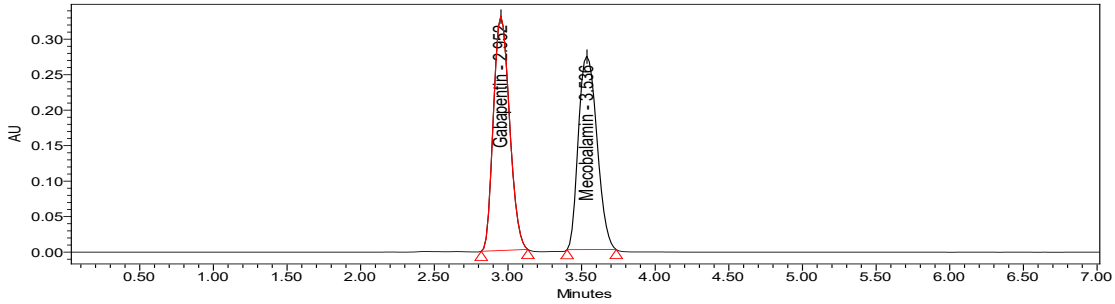


Fig 5: Chromatograms for accuracy (150%) standard 2

LINEARITY

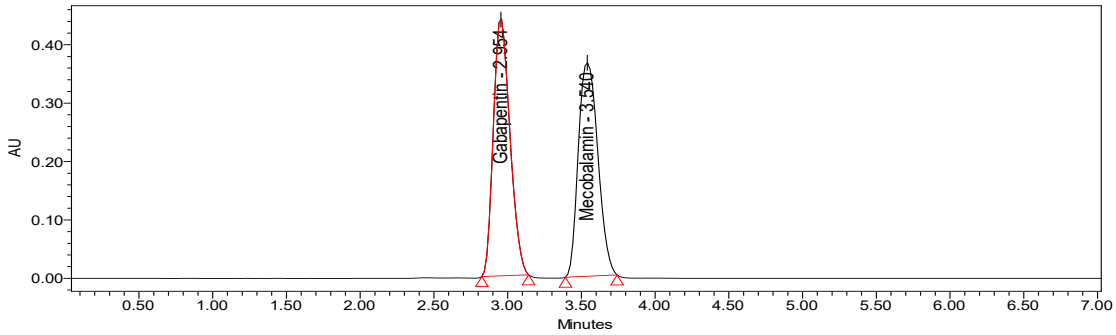


Fig-6: Chromatograms for 80 ppm

RUGGEDNESS

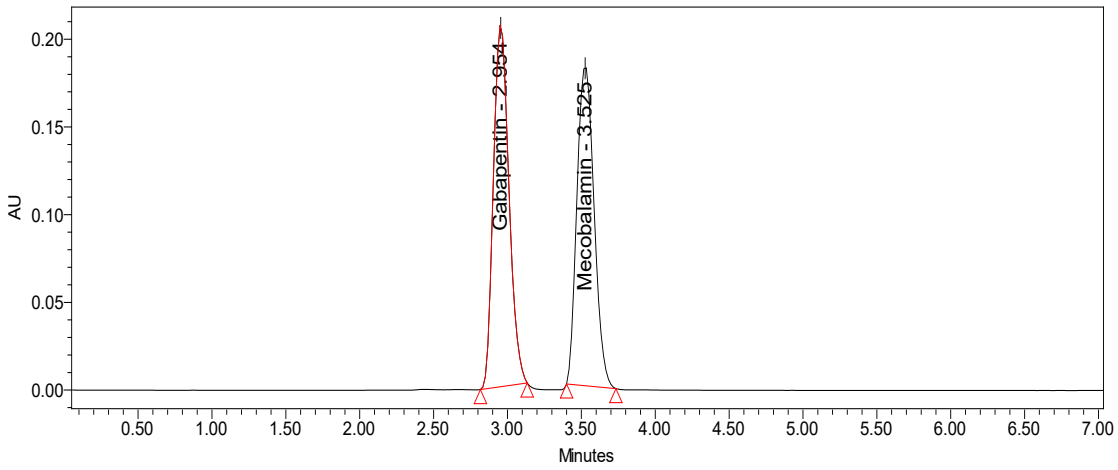


Fig. 7:

Chromatograms of system to system variability

ROBUSTNESS

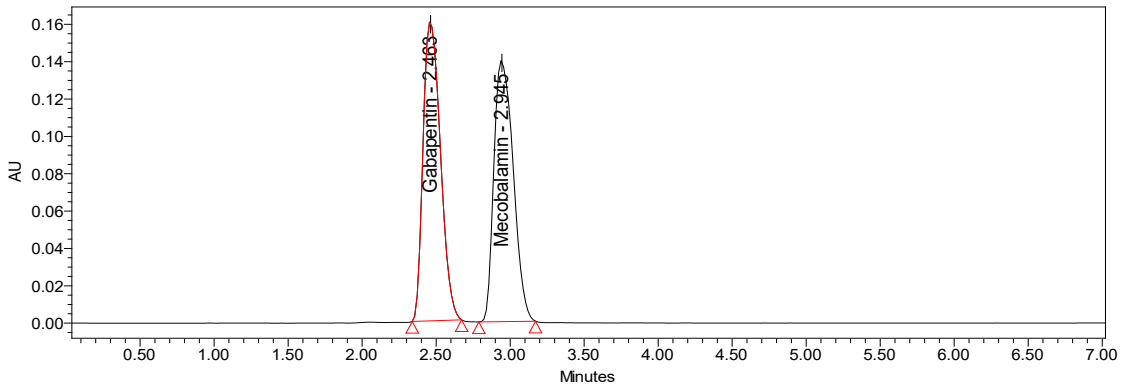


Fig-8: chromatograms for 1.2ml/min standard – 2

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ):

Gabapentin:

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 2431.578}{31282} = 0.25\ \mu g/ml$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$\frac{10 \times 2431.578}{}$$

$$= \frac{}{31282} = 0.77\ \mu g/ml$$

Mecobalamin:

$$LOD = \frac{3.3\ \sigma}{S} = \frac{3.3 \times 2124.413}{20193} = 0.34\ \mu g/ml$$

$$LOQ = \frac{10\ \sigma}{S} = \frac{10 \times 2124.413}{20193} = 1.05\ \mu g/ml$$

Discussion

The optimized RP-HPLC method using Methanol: Buffer (60:40 v/v) provided symmetrical peaks with acceptable resolution and theoretical plate count. The selected wavelength (271 nm) allowed simultaneous detection of both drugs with adequate sensitivity. Validation studies demonstrated excellent linearity ($r^2 = 0.999$), accuracy (98–102%), and precision (%RSD < 2%). Robustness studies under varied flow rates confirmed stability of the method. Compared to previously reported methods, the developed method offers reduced run time, economical mobile phase composition, and reliable quantification suitable for routine industrial application.

CONCLUSION

A simple, accurate, precise, and economical RP-HPLC method was successfully developed and validated for simultaneous estimation of Gabapentin and Methylcobalamin in tablet dosage form. The method complied with ICH Q2(R1) validation parameters and demonstrated excellent specificity, linearity, precision, and accuracy. Therefore, the developed method is suitable for routine quality control analysis in pharmaceutical industries.

REFERENCES

1) Snyder LR, Kirkland JJ. *Practical HPLC Method Development*. 2nd ed. Wiley; 1997.
2) Baheti KG, Galande VR. Simultaneous estimation of Gabapentin in presence of Methylcobalamin by HPTLC. Int J Res Pharm Biomed Sci. 2011.
3) Goti PP, Patel PB. Ratio derivative spectrophotometric method for estimation of Gabapentin and Methylcobalamin. J Pharm Res. 2013

4) ICH Harmonised Tripartite Guideline. Q2(R1): Validation of Analytical Procedures.
5) Yamada K. Role of Cobalt in Health and Disease. Springer; 2013