RP-HPLC Method Development And Validation For Simultaneous Estimation Of Amlodipine Besylate, Telmisartan And Bisoprolol Fumarate In Synthetic Mixture

Nidhi Jadav[1]*, Bhavisha Lukka[2], Mahek Kangad[3], Hetvi Garg[4], Shruti Sidapara[5], Archi Patel[6], Khushi Patel[7]

[1,2,3,4,5,6,7]*Department of Pharmaceutical Chemistry and Quality Assurance, Arihant school of Pharmacy & BRI, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat.*

Corresponding Author
Nidhi Jadav

Associate Professor, Pharmaceutical Chemistry and Quality Assurance, Arihant school of Pharmacy & BRI, Swarrnim Start-up & Innovation University, Gandhinagar, Gujarat. Email:nidhijadav.pharmacy@swarrnim.edu.in

ABSTRACT
The goal of the current study was to create and validate a straightforward, accurate, repeatable, and precise reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of Bisoprolol fumarate, Telmisartan, and Amlodipine besylate in a combination pharmaceutical dosage form. A C18 column with a mobile phase of buffer and acetonitrile (56:44, v/v) at a flow rate of 1.0 mL/min was used to accomplish chromatographic separation, and detection was carried out at 225 nm. The chromatographic conditions were optimized through systematic trials to obtain satisfactory resolution and peak characteristics. The developed method was validated according to ICH Q2 guidelines for specificity, linearity, precision, accuracy, limit of detection, limit of quantification, and robustness. The retention times of Bisoprolol fumarate, Amlodipine besylate, and Telmisartan were approximately 2.34, 4.18, and 15.03 min, respectively. The method exhibited excellent linearity with correlation coefficients greater than 0.998. Precision studies showed %RSD values below 2%, while recovery values ranged from 98% to 102%, confirming satisfactory accuracy. No interference from formulation excipients was observed, demonstrating good specificity. The method also remained robust under minor variations in chromatographic conditions. The validated RP-HPLC method is suitable for routine quality control analysis of these drugs in combined pharmaceutical formulations.
Keywords: RP-HPLC, Amlodipine Besylate, Telmisartan, Bisoprolol Fumarate, Method development, Method validation.

How to cite this article: Nidhi Jadav, Bhavisha Lukka, Mahek Kangad, Hetvi Garg, Shruti Sidapara, Archi Patel, Khushi Patel (2026 RP-HPLC Method Development And Validation For Simultaneous Estimation Of Amlodipine Besylate, Telmisartan And Bisoprolol Fumarate In Synthetic Mixture. International Journal of Drug Delivery Technology2026;16(74s): 660-666. DOI: 10.25258/ijddt.16.74s.79

INTRODUCTION

Hypertension is a severe chronic cardiovascular condition that poses a significant global public health concern due to its strong link to cardiovascular morbidity, premature mortality, and healthcare costs. Persistently elevated arterial blood pressure raises the risk of coronary artery disease, myocardial infarction, heart failure, stroke, chronic kidney disease, and other cardiovascular problems. Hypertension's multifactorial pathophysiology includes complicated interactions between genetic predisposition, aging, obesity, physical inactivity, high sodium consumption, bad eating choices, and environmental factors. Because hypertension is typically asymptomatic in its early stages, prompt diagnosis and good treatment are critical for avoiding long-term consequences.1

Amlodipine besylate, IUPAC name (Benzenesulfonic acid; 3- O-ethyl 5-Omethyl 2-(2-aminoethoxymethyl)-4-(2chlorophenyl)-6-methyl-1,4dihydropyridine-3,5-

dicarboxylate) with Molecular weight- 408.9 g/moL is a long-acting dihydropyridine CCB that lowers peripheral vascular resistance by preventing calcium input into vascular smooth muscle cells.2 Structure of Amlodipine besylate is represented in figure 1

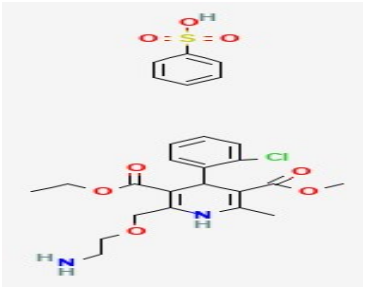

Figure 1. Structure of Amlodipine besylate

Telmisartan, IUPAC name (2-[4-[[4-methyl-6-(1-methylbenzimidazol- 2-yl)-2-propylbenzimidazol1yl] methyl]phenyl] benzoic acid) with Molecular weight- 514.6 g/moL is selectively inhibits angiotensin II AT_1 receptors, reducing vasoconstriction and aldosterone-mediated effects.3 Structure of Telmisartan is represented in figure 2.

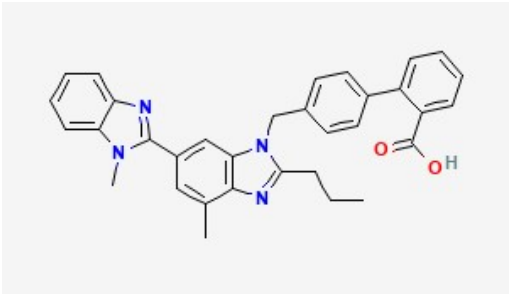

Figure 2. Structure of Telmisartan

Bisoprolol fumarate IUAC name (2E)-but-2-enedioic acid combined with bis(1-[(propan-2-yl)amino]-3-(4-{[2-(propan-2-yloxy)ethoxy]methyl}phenoxy)propan-2-ol) is a selective β_1-adrenergic receptor blocker, lowers heart rate, myocardial contractility, and cardiac output.4 These complementary pharmacological mechanisms provide a solid basis for exploring their combined use in hypertension therapy. Structure of Bisoprolol fumarate is represented in figure 3.

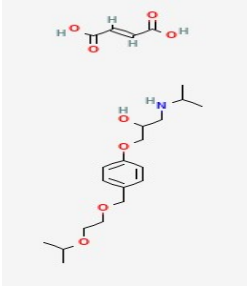

Figure 3. Structure of Bisoprolol fumarate

UV-visible spectrophotometry, HPLC, HPTLC, and other chromatographic techniques have all been used to determine these medicines singly or in binary combinations. Recent research have documented analytical methods for Telmisartan-Bisoprolol and other multidrug antihypertensive combinations; nevertheless, the literature on simple RP-HPLC methods for simultaneous estimation of Amlodipine besylate, Telmisartan, and Bisoprolol fumarate is still limited.5-13 As a result, the current work was conducted to develop and validate a simple, precise, accurate, robust, and reproducible RP-HPLC method for simultaneous estimation of these three drugs in a synthetic mixture using ICH guidelines for routine pharmaceutical quality control applications.

MATERIALS AND METHODS

Chemical and reagent

Amlodipine besylate (AML), Telmisartan (TEL), and Bisoprolol fumarate (BIS) were generously provided by Zydus Health Care Ltd., Sanand, India, and utilized exactly as received. Before analysis, the medicines' identities were validated using melting point determination and FT-IR spectroscopy. Methanol and acetonitrile of HPLC quality were acquired from Merck in India. Rankem Laboratories in India provided analytical reagent (AR)-grade potassium dihydrogen phosphate, sodium hydroxide, and hydrochloric acid, while Merck in India supplied hydrogen peroxide. Milli-Q water was used throughout the study to prepare solutions and the mobile phase.

Instrumentation

The Shimadzu LC-2010 CHT HPLC system (Shimadzu Corporation, Japan) was used for chromatographic analysis, with a PDA detector, a 100 µL fixed-loop injector, and LC Solution software for data acquisition and processing. Separation was achieved using a YMC Pack ODS-A (150 mm x 4.6 mm x 3 µ). UV spectrum analysis was carried out utilizing a Shimadzu UV-1800 double-beam UV-Visible spectrophotometer equipped with UV Probe software. FT-IR spectra were obtained using an Agilent Cary 630 FTIR spectrometer (Agilent Technologies, USA) and MicroLab Expert software. An analytical balance (Sartorius, Germany; sensitivity 0.001 g) was utilized to accurately weigh the materials. The pH of the mobile phase was adjusted with a Lab India digital pH meter, and sample solutions were sonicated using an Athena ultrasonic bath. A Lab Junction melting point.

Chromatographic conditions

Stationary phase: YMC Pack ODS-A (150 mm x 4.6 mm x 3 µ)

Mobile phase: Buffer: ACN (56:44%v/v)
Column Temperature: 30°C
Flow rate: 1.0 mL/Min
Wavelength: 225 nm
Run time: 22 minutes
Injection Volume: 10 µL

Preparation of buffer:
The buffer solution was made by adding 1.0 mL of Triethylamine (TEA) to 1000 mL of HPLC-grade water and adjusting the pH to 2.5 with diluted Orthophosphoric acid (OPA).

Preparation of Mobile Phase
The mobile phase was produced by combining the prepared buffer and acetonitrile in the ratio of 56:44 (v/v). Before use, the mixture was filtered through a 0.45 µm membrane filter and degassed via sonication. The prepared mobile phase was also utilized as a diluent throughout the experiment.

Selection of Wavelength
To determine the wavelength, reference solutions of amlodipine besylate, bisoprolol fumarate, and Telmisartan (20 µg/mL) were produced in methanol and scanned over the wavelength range of 200-400 nm using a UV-visible spectrophotometer. The superimposed UV spectra of the three drugs were analyzed to determine the best detection wavelength. Considering the 5:5:40 formulation ratio (amlodipine besylate: bisoprolol fumarate: Telmisartan), 225 nm was chosen as the analytical wavelength for subsequent RP-HPLC technique development and validation since it offered acceptable absorbance for all three analytes.

Preparation of Standard and Mixed Standard Solutions
Separate stock solutions of Amlodipine besylate (50 µg/mL), Bisoprolol fumarate (50 µg/mL), and Telmisartan (400 µg/mL) were prepared using methanol. Telmisartan was initially dissolved in 2 mL of 0.1 N HCl before being diluted with methanol, whereas Amlodipine besylate and Bisoprolol fumarate were dissolved directly in methanol. All solutions were sonicated for 15 minutes before diluting to volume. To develop and validate the RP-HPLC method, a mixed standard solution of 5 µg/mL amlodipine besylate, 5 µg/mL bisoprolol fumarate, and 40 µg/mL Telmisartan was generated by diluting the corresponding stock solutions with the diluent.

Preparation of Synthetic Mixture
For the preparation of synthetic mixture equivalent to 10 tablets, accurately weighed quantities of Telmisartan (400 mg), Amlodipine besylate (50 mg), and Bisoprolol fumarate (50 mg) were mixed with the excipients, namely Meglumine (150 mg), Sorbitol (800 mg), Microcrystalline Cellulose (1000 mg), Povidone (100 mg), Magnesium Stearate (20 mg), Corn Starch (500 mg), and Colloidal Silicon Dioxide (10 mg). The mixture was blended thoroughly using a mortar and pestle to obtain a homogeneous powder and stored in a tightly closed container until further use.

Method validation
The developed RP-HPLC method for the simultaneous estimation of Amlodipine besylate (AML), Bisoprolol fumarate (BIS), and Telmisartan (TEL) was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guideline. The method was evaluated for system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ) to establish its suitability for routine quality control analysis.

RESULTS AND DISCUSSION:
Method development:
The primary goal of this research is to develop and validate a RP-HPLC method for the concurrent analysis of AML, TEL and BIS in active pharmaceutical ingredients (API) and various formulations. The method is designed to comply with ICH standards while being simple, precise, reliable, efficient, and effective. To achieve this, several parameters were adjusted, including pH levels, elution procedures, flow rates, mobile phase composition, and the selection of suitable columns, as part of a refined and validated methodology. The optimization process focuses on producing distinct peaks, maximizing theoretical plate counts, minimizing tailing factors, and reducing analysis time to enhance the overall efficiency and effectiveness of the analytical procedure.14

System Suitability Test
The system suitability parameters were analyzed to assure the chromatographic system's reliability and accuracy. The results showed that the method met all of the needed criteria. The %RSD for replicate injections of the reference solution was less than 2.0, demonstrating that the procedure is precise. These results demonstrate that the system was appropriate for the analysis and met all performance requirements represented Table 1.

Table 1: System Suitability Data of AML, TEL and BIS

Parameters	AML	TEL	BIS
Retention time (Rt)	4.18	15.02	2.34
Resolution	2.0	9.6	–
Theoretical Plates(N)	8410	16091	5185
Tailing factor (Tf)	1.26	1.01	1.4

Specificity

The investigation confirmed that no excipients or other possible components interfered with the drugs peaks. This is verified by the spectral data, which confirms the method's capacity to identify and quantify analytes in the presence of other compounds.

Linearity and Range

The linearity of all three drugs was evaluated by preparing standard solutions at five different concentration levels ranging from 2.50–7.50 ppm (50–150%).The peak areas obtained were plotted against corresponding concentrations to construct the calibration curve. The overlay chromatogram was represented in Figure 4. The correlation coefficients greater than 0.997 indicate excellent linearity shown in Table 2. and confirm that the developed method is suitable for quantitative estimation of the drugs.

Table 2 Calibration Curve data

Parameter	AML	TEL	BIS
Slope	59652.96	292829.47	94712.48
Intercept	6196.20	139996.40	15428.60
Correlation coefficient (R^2)	0.99822	0.99812	0.99976

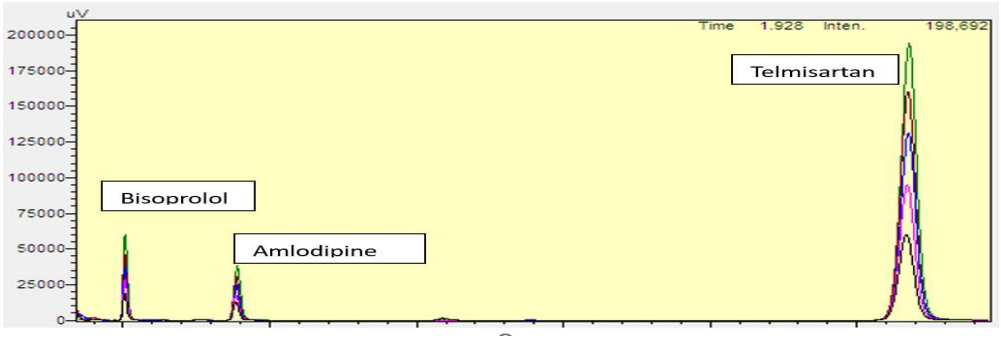

Figure 4: Overlay chromatogram of all three drugs

LOD and LOQ

LOD and LOQ value was obtained by calculating standard deviation of each calibration standard. The result of LOD and LOQ value represented in Table 3.

Table 3: Result of LOD & LOQ

Parameter	AML	TEL	BIS
LOD	0.710	3.339	0.260
LOQ	2.153	10.120	0.788

Precision

The precision was effectively achieved within the limit, as the total percent RSD of the repeatability, intraday and interday precision were less than 2. Result of repeatability is shown in Table 4.

Table 4: Result of Repeatability

Sets	AML	TEL	BIS
1	173904	2467647	177461
2	173881	2463042	177619
3	174229	2464389	177748
4	174291	2466472	177975
5	174383	2491445	181591
6	175341	2497112	178492
Mean	174338	2475018	178481
% RSD	0.3	0.6	0.9

Accuracy

The accuracy of the developed RP-HPLC method for the simultaneous estimation of AML, BIS, and TEL was evaluated by recovery studies using the standard addition method. Pre-analyzed sample solutions were spiked with known amounts of the respective standard solutions at three concentration levels, namely 50%, 100%, and 150% of the target concentration. The percentage recovery of AML, BIS, and TEL was calculated at each level. The results of the recovery studies are presented in Table 5.

Table 5: Result of Accuracy

Drug	Accuracy Level	% Recovery (n = 1)	% Recovery (n = 2)	% Recovery (n = 3)	Mean % Recovery	% RSD
AML	50%	101.60	102.00	100.80	101.5	0.6
	100%	100.60	98.00	100.60	99.7	1.5
	150%	98.70	99.70	97.30	98.6	1.2
TEL	50%	98.40	99.00	98.00	98.5	0.5
	100%	98.00	100.50	98.90	99.1	1.3
	150%	100.60	101.30	99.10	100.3	1.1
BIS	50%	101.60	100.80	101.20	101.2	0.4
	100%	100.40	100.20	100.60	100.4	0.2
	150%	99.20	100.30	97.60	99.0	1.4

Robustness

In robustness study deliberate change in parameters such as Flow rate, mobile phase and Temperature resulted in less than 2% Relative Standard Deviation of peak area, proving the method's robustness. The results of the robustness are presented in Table 6.

Table 6: Result of Robustness

Condition	AML Mean	%RSD	TEL Mean	%RSD	BIS Mean	%RSD
Temp. 20°C	190157	0.0	2606759	0.0	195831	0.1
Temp. 30°C	190660	0.4	2615070	0.1	196122	0.4
Flow 0.9 mL/min	210361	0.1	2884357	0.1	216498	0.1
Flow 1.1 mL/min	172857	0.2	2376144	0.5	180522	0.2
Mobile Phase (58:42)	189741	0.1	2599313	0.1	195886	0.5
Mobile Phase (54:46)	189636	0.1	2616932	0.1	195138	0.0

Analysis of Synthetic mixture

All values are within the acceptable limit of 95–105% as per ICH Guidelines Shown in Table 7. The close agreement between actual and obtained concentrations confirms that excipients (placebo) do not interfere with the estimation of the drugs. So the developed RP-HPLC method is accurate, precise, and specific in the presence of placebo, and can be successfully applied for routine quality control analysis of combined pharmaceutical dosage forms.

Table 7: Result of assay

	% Assay (n=3)			%RSD		
Synthetic Mixture	AML	TEL	BIS	AML	TEL	BIS
	98.1	100.5	101.8	0.0	0.2	0.3

SUMMARY AND CONCLUSION

The RP-HPLC method developed and validated for the simultaneous estimation of amlodipine besylate, Telmisartan and Bisoprolol fumarate in a synthetic mixture was found to be accurate, precise and robust. Under optimal chromatographic conditions, the method successfully separated both drugs with well- defined peaks. The validation parameters, which included Linearity, Accuracy, Precision, LOD, LOQ, and Robustness, complied with ICH Q2 (R2) guidelines, proving the method's reliability for routine analyses shown in Table 8. Overall, our RP-HPLC method is simple, sensitive, and reproducible, making it a valuable tool for quality control and stability studies of Amlodipine besylate, Telmisartan and Bisoprolol fumarate in pharmaceutical formulations

Table 8: Validation summary data

Validation parameter	AML	TEL	BIS
Specificity	No interference	No interference	No interference
Slope	59652.96	292829.47	94712.48
Intercept	6196.20	139996.40	15428.60
Correlation coefficient (R^2)	0.99822	0.99812	0.99976
Intra-day Precision	0.18-1.75	1.03-1.84	0.05-1.90
Inter-day Precision	0.82-1.08	0.31-0.48	0.31-1.65
LOD	0.710	3.339	0.260
LOQ	2.153	10.120	0.788
Accuracy (Recovery)	0.4-1.1	0.5-1.2	0.6-1.4
Robustness	0.1-0.50%	0.1-0.60%	0.1-1.0%
Assay of Marketed Formulation (% label Claim ±SD)	98.1%	100.5%	101.8%

Acknowledgment

The Authors would like to thank Arihant School of Pharmacy & BRI, Gandhinagar, Gujarat and Analyze Analytical Lab and Research Centre for providing all facilities performing experimental work.

REFERENCES

1. Carey RM, Moran AE, Whelton PK. Treatment of hypertension: a review. Jama. 2022 Nov 8;328(18): 1849-61.doi:10.1001/jama.2022.19590.

2. Indian Pharmacopoeia Commission. (2022). Amlodipine besylate / related monographs. In Indian Pharmacopoeia (Vol. II, pp. 1457–1458). Ministry of Health and Family Welfare, Government of India.

3. Indian Pharmacopoeia Commission. (2022). Telmisartan. In Indian Pharmacopoeia (Vol. II, pp. 2049–2051). Ministry of Health and Family Welfare, Government of India.

4. United States Pharmacopeial Convention. Bisoprolol fumarate. In: United States Pharmacopeia–National Formulary. Rockville (MD): USP; 2011.

5. Dalal J, Guha S, Reddy YV, Ponde CK, Shrivastava S, Badani R, Joshi S, Pande A, Kumar V, Bhalla N, Pradhan A. Telmisartan Plus Amlodipine as the Preferred Initial Combination in Newly Diagnosed Indian Patients with Hypertension: An Expert Consensus Statements. The Journal of the Association of Physicians of India. 2023 Dec 1;71(12):56-61.

6. Sattar OI, Abuseada HH, Ramzy S, Abuelwafa MM. Three spectrophotometric quantitative analysis of Bisoprolol fumarate and Telmisartan in fixed-dose combination utilizing ratio spectra manipulation methods. Scientific reports. 2024 Oct 2;14(1):22899.doi:10.1038/s41598-024-72525-6.

7. Bhavsar A, Tandel D, Patel K, Parmar R, Patel G, Gandhi T. DoE assisted, stability indicating HPTLC method development and validation for concurrent determination of Telmisartan and Bisoprolol fumarate in tablet dosage form. Essential Chem. 2025 Dec 31;2(1):1-6.

8. Mistry RP, Shah C, Jat R. RP-HPLC method development and validation for simultaneous estimation of Telmisartan, rosuvastatin calcium, and Amlodipine besylate in combination. Folia Medica. 2022 Feb 28;64(1):103-9.

9. Shukla S, Patel R. Development of Stability Indicating Greener Rp-Hplc Method for the Analysis of Drugs Used for Combined Hypertension Therapy in Pharmaceutical Preparations Using Analytical Quality by Design. World. 2026;5(1).

10. Kammoun AK, Khedr A, Fawzy MG, Kamel EB. Merging green chemistry with spectrophotometry: Univariate vs. multivariate assessment of a novel triple antihypertensive combination, amlodipine, Telmisartan, and indapamide. Results in Chemistry. 2026 Feb 27:103192.

11. Vemula K, Ponnuswamy NK, Raju B. Method Development and Validation For Simultaneous Estimation of Amlodipine besylate and Telmisartan in Bulk and Pharmaceutical Formulation by RP-HPLC. World Journal of Pharmaceutical Research. 2019 Sep 25;8:937-48.

12. Rakesh, D., & Babu, M. A. (2023). Development and validation of a novel RP-HPLC method for simultaneous determination of Telmisartan and amlodipine in tablet formulation. Indo American Journal of Pharmaceutical Sciences, 10(3), 118–126.

13. ICH Q2(R1): Validation of Analytical Procedures – Text and Methodology. (2005). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

14. Sudhansu Ranjan Swain, Mandeep Kumar Gupta, Bhuvnesh Kumar Singh, Anshika Bhatnagar, Surya Nath Pandey. RP-HPLC Method Development and Validation for Simultaneous Estimation of Metformin, Dapagliflozin and Glimepiride in fixed dose Combinations. Research Journal Pharmacy and Technology. 2026;19(5):2267-2. doi: 10.52711/0974-360X.2026.00326