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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF CILNIDIPINE AND NEBIVOLOL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:
A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Cilnidipine and Nebivolol in tablet dosage form. Chromatographic separation was achieved using a Hypersil BDS C18 column (150 mm x 4.6 mm, 5 µm) with a mobile phase consisting of acetate buffer and acetonitrile in the ratio of 30:70 v/v. The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 256 nm. The retention times for Cilnidipine and Nebivolol were found to be 2.3 and 2.8 minutes, respectively. The method showed good linearity in the range of 5–30 µg/mL for Cilnidipine and 2.5–15 µg/mL for Nebivolol, with correlation coefficients of 0.999. The method was validated as per ICH Q2(R1) guidelines for precision, accuracy, linearity, robustness, LOD, and LOQ. The proposed method can be successfully applied for routine quality control of the cited drugs in pharmaceutical formulations.

Keywords: Cilnidipine, Nebivolol, RP-HPLC, Method Validation, ICH Guidelines

INTRODUCTION

Hypertension is a chronic medical condition affecting a significant portion of the global population and is a major risk factor for cardiovascular diseases such as stroke, myocardial infarction, and heart failure. To manage this condition effectively, combination therapy involving two or more antihypertensive agents is commonly employed. Cilnidipine and Nebivolol are one such combination widely prescribed for the treatment of hypertension due to their complementary mechanisms of action.

Cilnidipine is a fourth-generation dihydropyridine calcium channel blocker that exhibits dual inhibition of L-type and N-type calcium channels. This dual action not only reduces peripheral vascular resistance through vasodilation but also inhibits sympathetic nerve activity, leading to better control of blood pressure and reduced risk of reflex tachycardia. Nebivolol, on the other hand, is a selective beta-1 adrenergic blocker known for its vasodilatory effect mediated through nitric oxide release. It decreases cardiac output and suppresses renin activity, which further contributes to blood pressure reduction.

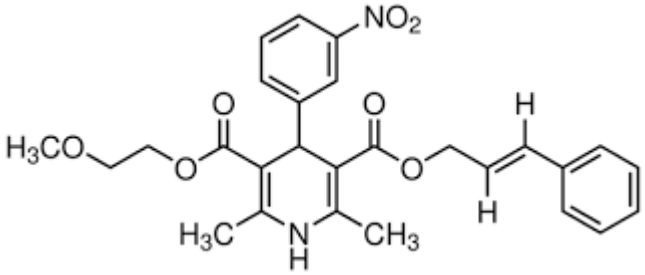

Fig. 1. Chemical Structure of Cilnidipine

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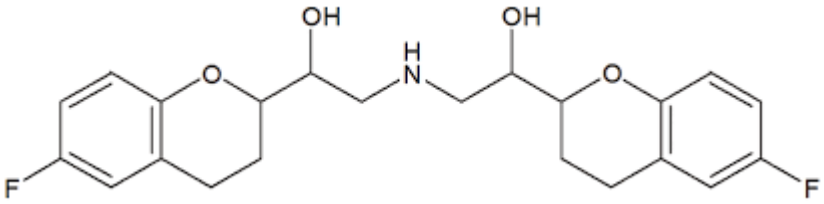

Fig. 2. Chemical Structure of Cilnidipine

The fixed-dose combination of Cilnidipine and Nebivolol is advantageous because it enhances therapeutic efficacy, improves patient adherence, and minimizes side effects compared to monotherapy. Given their clinical significance, ensuring the quality, safety, and efficacy of this combination in pharmaceutical dosage forms is essential. Reliable analytical methods are therefore required for the accurate and precise quantification of these drugs.

High-performance liquid chromatography (HPLC) is one of the most preferred analytical techniques for the simultaneous estimation of pharmaceutical compounds due to its sensitivity, accuracy, and reproducibility. While several analytical methods have been reported for individual drugs, there is limited literature on the simultaneous estimation of Cilnidipine and Nebivolol using RP-HPLC. A validated RP-HPLC method not only supports regulatory compliance but also facilitates routine quality control during manufacturing.

The objective of this study was to develop and validate a rapid, sensitive, and cost-effective RP-HPLC method for the simultaneous quantification of Cilnidipine and Nebivolol in combined tablet dosage form. The method was developed with a focus on achieving optimal resolution, short run time, and adherence to ICH Q2(R1) validation parameters, making it suitable for application in quality control laboratories and pharmaceutical industries.

Materials and Methods

Chemicals and Reagents: Cilnidipine and Nebivolol reference standards, Cilacar NB 5 tablets, acetonitrile, acetate buffer, glacial acetic acid, methanol, and HPLC-grade water.

Instrumentation: WATERS HPLC 2965 system with PDA detector; Empower 2 software; UV-Vis spectrophotometer (PG Instruments T60).

Preparation of Buffer and Diluent: Acetate buffer was prepared using sodium acetate and glacial acetic acid, pH adjusted to 4.5. The diluent was prepared by mixing water and methanol in a 1:1 ratio.

Preparation of Standard Solutions: Accurately weighed 10 mg of Cilnidipine and 5 mg of Nebivolol were dissolved in diluent and diluted to obtain 20 µg/mL and 10 µg/mL working solutions, respectively.

Preparation of Sample Solution: Powdered tablet equivalent to one dose was dissolved in diluent, sonicated, filtered, and diluted to obtain the target concentrations.

Validation Parameters: The method was validated for system suitability, specificity, linearity, precision, accuracy, robustness, LOD, and LOQ as per ICH guidelines.

Results and Discussion

The RP-HPLC method for the estimation of Cilnidipine and Nebivolol was developed using a Hypersil BDS C18 column (150 mm × 4.6 mm, 5 µm) as the stationary phase, which provided effective separation and peak symmetry. The mobile phase consisted of acetate buffer and acetonitrile in a ratio of 30:70 v/v, selected to achieve optimal resolution and retention time for both analytes. The flow rate was maintained at 1.0 mL/min, ensuring reproducible elution and minimal baseline noise. Detection was carried out at a wavelength of 256 nm, at which both drugs exhibit significant absorbance, ensuring adequate sensitivity. The column oven temperature was set at 30°C to maintain consistency in retention times, and a sample injection volume of 10 µL was used for all analyses. These conditions were optimized to ensure rapid, accurate, and reproducible quantification of the drugs in pharmaceutical dosage forms.

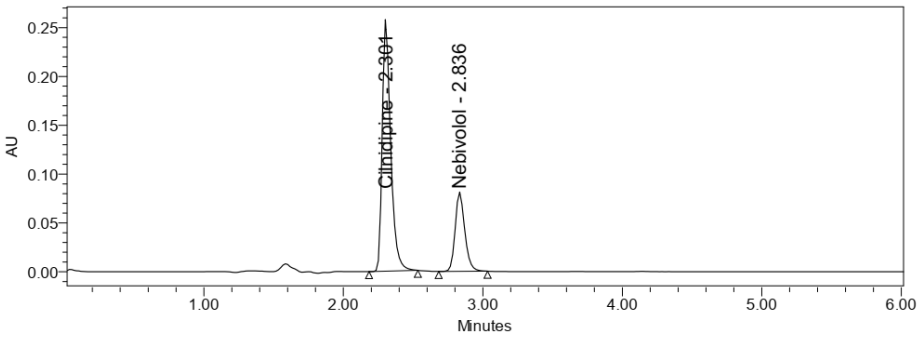

Fig 3 Optimized chromatogram of Cilnidipine and Nebivolol

System Suitability: The retention times were 2.3 min for Cilnidipine and 2.8 min for Nebivolol. Theoretical plates (>2000), tailing factors (<2), and %RSD (<2%) met ICH requirements.

Table: 1 System suitability studies of Cilnidipine and Nebivolol method

Property	Cilnidipine	Nebivolol
Retention time (t_R)	2.3 min	2.8 min
Theoretical plates (N)	5466	248
Tailing factor (T)	1.32	1.21

Linearity: Linear responses were observed for Cilnidipine (5–30 µg/mL) and Nebivolol (2.5–15 µg/mL) with correlation coefficients of 0.999. Regression equations were y = 47355x + 5833.6 for Cilnidipine and y = 32770x + 581.68 for Nebivolol.

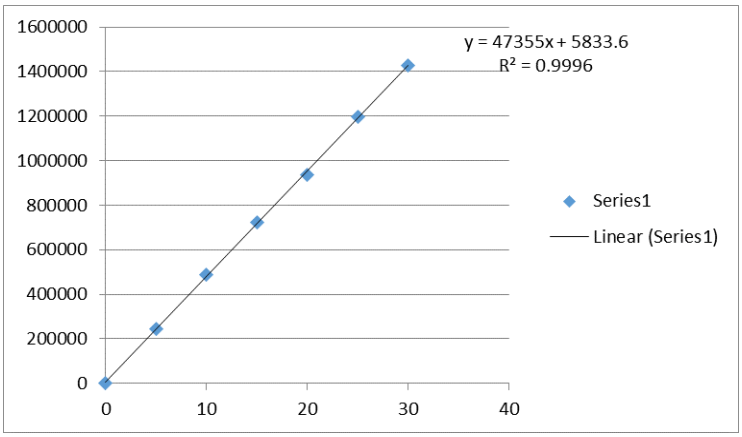

Fig: 4 Calibration curve of Cilnidipine

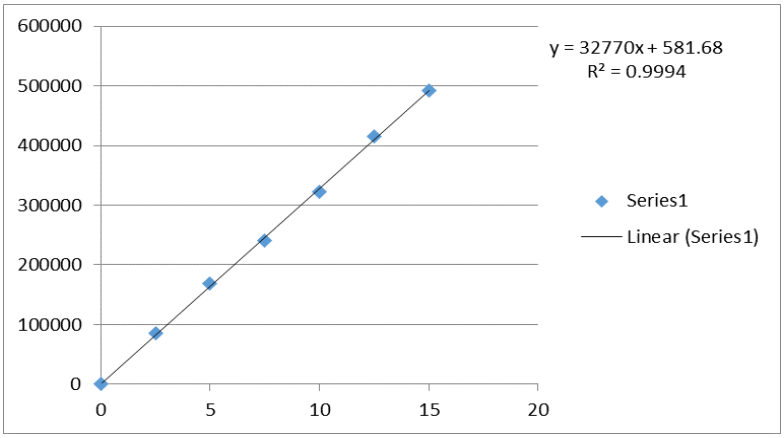

Fig: 5 Calibration curve of Nebivolol

Precision: %RSD for repeatability and intermediate precision was <2% for both drugs, confirming method reproducibility.

Accuracy: Recovery studies at 50%, 100%, and 150% levels showed recoveries between 98.4% and 100.77%.

Table: 2 Accuracy results of Cilnidipine and Nebivolol

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	Avg
Cilnidipine	10	9.85	98.5	98.4
	20	19.26	96.3	
	30	30.12	100.4	
Nebivolol	5	5.11	102.2	100.77
	10	9.86	98.6	
	15	15.23	101.53	

LOD and LOQ: LOD was 0.4 µg/mL for Cilnidipine and 0.1 µg/mL for Nebivolol. LOQ was 1.2 µg/mL and 0.3 µg/mL, respectively.

Robustness: Small changes in flow rate, mobile phase composition, and temperature did not significantly affect system performance.

Assay: The % assay values were found to be 99.39% for Cilnidipine and 99.83% for Nebivolol, indicating compliance with pharmacopeial standards.

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cilnidipine and Nebivolol in Tablet dosage form. Chromatogram was run through Hypersil BDS (150mm 4.6mm, 5µ). Mobile phase containing Acetate Buffer and Acetonitrie in the ratio of 30: 70A was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Cilnidipine and Nebivolol was 256 nm. Retention time of Cilnidipine and Nebivolol were found to be 2.3min and 2.8 min. %RSD of the Cilnidipine and Nebivolol were and found to be 1.1 and 1.6, respectively. %Recover was Obtained as 98.4 and 100.77 for Cilnidipine and Nebivolol respectively. LOD, LOQ values are obtained from regression equations of Cilnidipine and Nebivolol were 0.4, 0.1 and 1.2, 0.3 respectively. Regression equation of Cilnidipine is y = 47355x + 5833.6 and of Nebivolol is y = 32770x + 581.68. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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