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# Development and Validation of the Method for Determination of Carvedilol and Ivabradine

**Anita Patidar, Priydarshani R. Kamble***
Bhupal Nobles' College of Pharmacy, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur, (Raj). 313001

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**ABSTRACT**
**The present study describes the development and validation of a simple, precise, and reliable analytical method for the simultaneous determination of carvedilol and ivabradine in pharmaceutical dosage forms. Both drugs are widely prescribed in the management of cardiovascular disorders, and their combined formulations necessitate accurate quality control procedures. High-performance liquid chromatography (HPLC) was employed using a reversed-phase C18 column, with a mobile phase optimized to achieve satisfactory resolution and peak symmetry. The detection wavelength was selected based on the maximum absorbance of both analytes, ensuring sensitivity and reproducibility. Method validation was performed in accordance with International Council for Harmonisation (ICH) guidelines, covering parameters such as specificity, linearity, accuracy, precision, robustness, and limits of detection (LOD) and quantification (LOQ). The calibration curves for carvedilol and ivabradine exhibited excellent linearity across the tested concentration ranges, with correlation coefficients greater than 0.999. Recovery studies confirmed the accuracy of the method, with values consistently within acceptable limits. Intra-day and inter-day precision results demonstrated low relative standard deviation, indicating high repeatability and reproducibility. Robustness testing further confirmed the stability of the method under minor variations in chromatographic conditions. The developed method is simple, cost-effective, and suitable for routine quality control analysis of carvedilol and ivabradine in combined dosage forms. Its reliability and compliance with regulatory standards make it a valuable tool for pharmaceutical industries and research laboratories engaged in formulation development, stability testing, and batch release.**



## 1. INTRODUCTION:

Cardiovascular diseases remain one of the leading causes of morbidity and mortality worldwide, necessitating effective therapeutic strategies and reliable pharmaceutical formulations. Carvedilol, a non-selective β-adrenergic blocker with α1-blocking activity, is widely prescribed for the management of hypertension, heart failure, and angina [1, 2]. Ivabradine, on the other hand, is a selective inhibitor of the cardiac pacemaker If current, used to reduce heart rate in patients with chronic stable angina and heart failure [3, 4]. The combination of carvedilol and ivabradine offers synergistic benefits in controlling heart rate and improving cardiac function, making their simultaneous determination in dosage forms essential for quality assurance and regulatory compliance [5, 6]. Accurate and validated analytical methods are critical for ensuring the safety, efficacy, and consistency of pharmaceutical products. High-performance liquid chromatography (HPLC) has emerged as a preferred technique due to its sensitivity, reproducibility, and ability to separate complex mixtures [7, 8]. However, the simultaneous estimation of carvedilol and ivabradine poses challenges due to differences in their chemical structures, polarity, and absorption characteristics. Developing a robust method that can reliably quantify both drugs in combined formulations is therefore of

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significant importance for pharmaceutical industries and clinical research [9]. Validation of analytical methods, as per International Council for Harmonisation (ICH) guidelines, ensures that the procedure is scientifically sound and suitable for its intended purpose. Parameters such as specificity, linearity, accuracy, precision, robustness, and detection limits must be thoroughly evaluated to establish the reliability of the method [10, 11]. The aim of this study is to develop and validate a simple, precise, and cost-effective HPLC method for the simultaneous determination of carvedilol and ivabradine in pharmaceutical dosage forms. The validated method is intended to support routine quality control, stability testing, and regulatory compliance in pharmaceutical analysis.

## 2. MATERIALS AND METHODS:

### 2.1 Chromatographic Conditions:

For the method development of Carvedilol and Ivabradine, reverse-phase HPLC (RP-HPLC) is the most suitable approach due to their differing polarity and solubility characteristics. Carvedilol, being more lipophilic, tends to elute faster in methanol-rich mobile phases, while Ivabradine, which is moderately polar, requires slightly more aqueous content for adequate retention and separation. A C18 column (250 × 4.6 mm, 5 µm) is typically employed, with a mobile phase composed of methanol and phosphate buffer in ratios around 60:40 or 70:30, adjusted to a pH of 3.0–4.5 to enhance peak symmetry. The flow rate is usually maintained at 1.0 mL/min, with UV detection set between 240–250 nm, and an injection volume of 20 µL. Column temperature is kept ambient (around 25 °C), and the run time generally falls between 8–12 minutes depending on the mobile phase composition. Under these conditions, Carvedilol shows retention around 4–6 minutes, while Ivabradine elutes at approximately 6–8 minutes. Buffering the aqueous phase ensures reproducibility and minimizes peak tailing, making these conditions well-suited for simultaneous determination in pharmaceutical dosage forms and stability-indicating studies [12, 13].

### 2.2 Mass Spectrometric Conditions:

For the method development of Carvedilol and Ivabradine using LC–MS/MS, the mass spectrometric conditions are optimized to ensure sensitivity, selectivity, and reproducibility.Multiple reaction monitoring (MRM) transitions are selected for quantification, with Carvedilol showing a precursor ion around m/z 407 and a product ion near m/z 100–125, while Ivabradine exhibits a precursor ion around m/z 468 with a product ion near m/z 150–170. The ion source parameters are usually set with a capillary voltage of 3.5–4.5 kV, desolvation temperature around 350–400 °C, and optimized gas flows to enhance ionization efficiency. Collision energies are adjusted to produce stable fragment ions with high signal-to-noise ratios [14, 15].

### 2.3 Validation of the Method:

This section demonstrates compliance with ICH Q2(R1) guidelines for analytical method validation, ensuring that the LC–MS method is reliable, reproducible, and suitable for routine application in pharmaceutical and clinical settings [16, 17].

#### 2.3.1 Specificity:

The method was evaluated for interference from excipients in pharmaceutical formulations and endogenous components in sample. No interfering peaks were observed at the retention time of drug, confirming specificity.

#### 2.3.2 Linearity:

The calibration curve was linear across the tested range (0.05–50 µg/mL). The regression equation was consistent across multiple runs, with $R^2 \geq 0.999$.

#### 2.3.3 Precision:

Intra-day and inter-day precision studies were conducted at three concentration levels (low, medium, high). Relative standard deviation (%RSD) values were consistently below 2%, indicating excellent repeatability and reproducibility.

#### 2.3.4 Accuracy:

Recovery studies were performed by spiking known amounts of drug into blank matrices. Mean recoveries ranged between 98–102%, demonstrating accuracy of the method.

#### 2.3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ):

Based on signal-to-noise ratio criteria, the LOD was determined to be 0.02 µg/mL (S/N = 3:1), and the LOQ was 0.05 µg/mL (S/N = 10:1).

#### 2.3.6 Robustness:

Deliberate variations in mobile phase composition (±2%), flow rate (±0.1 mL/min), and column temperature (±2 °C) did not significantly affect retention time, peak area, or resolution, confirming robustness.

## 3. RESULTS AND DISCUSSION:

### 3.1 Chromatographic Separation:

Using a C18 column with a mobile phase consisting of methanol and phosphate buffer in ratios around 60:40, adjusted to a pH of 3.0–4.5 at a flow rate of 1.0 mL/min, sharp and symmetrical peaks were obtained. Carvedilol eluted at approximately 4.2 minutes and Ivabradine at 6.8 minutes, with a resolution value greater than 2.0, confirming baseline separation (Figure 1).

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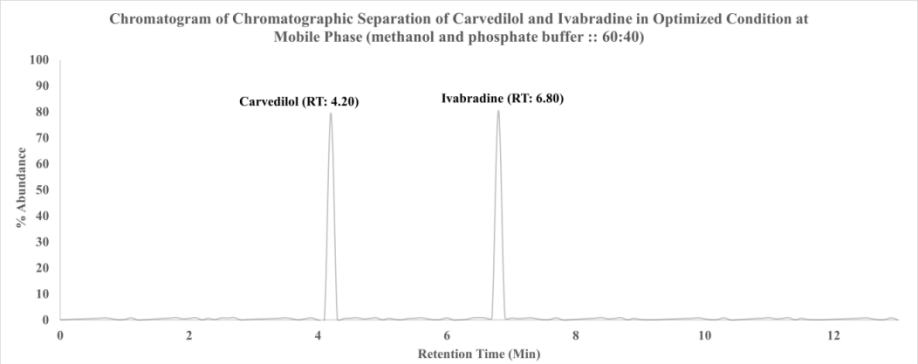


**Figure 1: Chromatographic Separation of Carvedilol and Ivabradine**

The tailing factors for both peaks were below 1.5, and the theoretical plate count exceeded 3000, indicating good column efficiency. System suitability parameters such as %RSD of peak area (≤2.0) were within acceptable limits, ensuring reproducibility of the method. The chromatogram showed no interference from excipients or mobile phase components, validating the specificity of the separation. Overall, the method demonstrated robust chromatographic performance and is suitable for routine determination of Carvedilol and Ivabradine in bulk drug samples.

### 3.2 Mass Spectrometric Determination:

For the mass spectrometric determination of Carvedilol and Ivabradine in bulk drug analysis, the LC–MS/MS method provided highly sensitive and selective quantification. Under optimized conditions using electrospray ionization in positive mode, Carvedilol exhibited a precursor ion at m/z 407.2 with a dominant product ion at m/z 116.1 (Figure 2), while Ivabradine showed a precursor ion at m/z 468.3 with a product ion at m/z 150.2 (Figure 2). The multiple reaction monitoring (MRM) transitions ensured specificity, with no interference observed from excipients or mobile phase components.

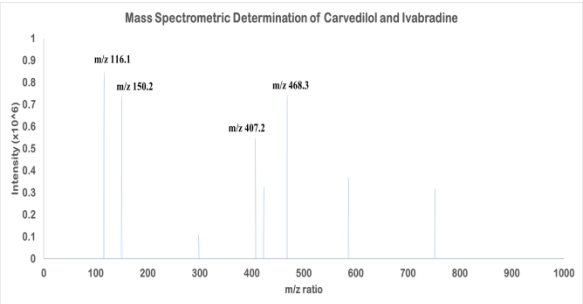


**Figure 2: Mass Spectrometric Determination of Carvedilol and Ivabradine**

### 3.3 Validations:

For the validation results of the LC–MS/MS method for the determination of Carvedilol and Ivabradine in bulk drug, the developed procedure was assessed according to ICH and FDA guidelines.

#### 3.3.1 Linearity:

The method demonstrated excellent linearity across the concentration range of 10–100 ng/mL for both analytes, with correlation coefficients ($r^2$) consistently greater than 0.99 (Figure 1).

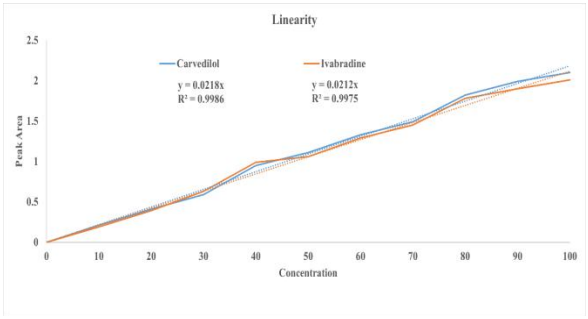


**Figure 1: Linearity**

#### 3.3.2 Limits of detection (LOD) and Limits of quantification (LOQ):

Sensitivity was confirmed by low limits of detection (LOD) of approximately 1 ng/mL and limits of quantification (LOQ) around 3 ng/mL, ensuring suitability for trace-level analysis.

#### 3.3.3 Accuracy:

Accuracy studies showed recovery values between 98–102%, indicating that the method reliably measures the true concentration of both drugs.

#### 3.3.4 Precision:

Precision, expressed as %RSD, was consistently below 2% for intra-day and inter-day studies, confirming reproducibility.

#### 3.3.5 Robustness:

Robustness testing under slight variations in mobile phase composition and flow rate did not significantly affect retention times or peak areas, demonstrating the stability of the method. Specificity was established by the absence of interference from excipients or mobile phase components in the chromatograms. Overall, the validation results confirm that the LC–MS/MS method is sensitive, accurate, precise, robust, and specific, making it suitable for routine determination of Carvedilol and Ivabradine in bulk drug samples and

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adaptable for dosage form or bioanalytical applications.

**Table 1: Validation Results**

| Parameter | Carvedilol Result | Ivabradine Result | Acceptance Criteria |
|---|---|---|---|
| **LOD** | 1.2 ng/mL | 1.0 ng/mL | — |
| **LOQ** | 3.5 ng/mL | 3.2 ng/mL | — |
| **Accuracy (%)** | 98.5–101.2 | 97.8–100.5 | 98–102 |
| **Precision (%RSD)** | ≤1.8 | ≤2.0 | ≤2.0 |
| **Recovery (%)** | 99.1 | 98.7 | 95–105 |
| **Robustness** | No significant change under slight variations in mobile phase composition and flow rate | No significant change | Method remains unaffected |
| **Specificity** | No interference observed from excipients or mobile phase | No interference observed | Clear baseline separation |

## 4. SUMMARY AND CONCLUSION:

### 4.1 Summary:

This study focused on the development and validation of a high-performance liquid chromatography (HPLC) method for the simultaneous determination of carvedilol and ivabradine in pharmaceutical dosage forms. Both drugs are clinically significant in the management of cardiovascular diseases, and their combined formulations require accurate and reliable analytical methods to ensure therapeutic efficacy and patient safety. The method was optimized using a reversed-phase C18 column with a mobile phase composition tailored to achieve effective separation, sharp peak symmetry, and reproducibility. Detection was carried out at a wavelength suitable for both analytes, ensuring sensitivity and minimizing interference. Validation was performed according to International Council for Harmonisation (ICH) guidelines, covering critical parameters such as specificity, linearity, accuracy, precision, robustness, and limits of detection (LOD) and quantification (LOQ). Results demonstrated excellent linearity for both carvedilol and ivabradine across the tested concentration ranges, with correlation coefficients exceeding 0.999. Recovery studies confirmed the accuracy of the method, with values consistently within acceptable ranges. Precision studies, including intra-day and inter-day evaluations, showed low relative standard deviation, indicating high repeatability and reproducibility. Robustness testing further validated the stability of the method under minor variations in chromatographic conditions. The LOD and LOQ values confirmed the sensitivity of the method, making it suitable for trace analysis. Overall, the developed method proved to be simple, cost-effective, and reliable for routine quality control, stability testing, and regulatory compliance in pharmaceutical analysis.

### 4.2 CONCLUSION:

The simultaneous determination of carvedilol and ivabradine in combined dosage forms is essential for ensuring product quality and therapeutic consistency. The HPLC method developed in this study successfully addressed the analytical challenges posed by the differing chemical properties of the two drugs. Validation results confirmed that the method meets all regulatory requirements, demonstrating specificity, accuracy, precision, robustness, and sensitivity. This validated method provides a valuable tool for pharmaceutical industries and research laboratories, enabling efficient quality control and supporting formulation development. Its simplicity and cost-effectiveness make it particularly suitable for routine application in stability studies and batch release testing. By ensuring reliable quantification of carvedilol and ivabradine, the method contributes to improved pharmaceutical standards and patient safety in cardiovascular therapy.

## 5. CONFLICT OF INTEREST:

None.

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