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# Quality By Design Approach In HPLC Method Development and Validation for Simultaneous Determination of Carvedilol and Ivabradine with Greenness Assessment

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**Abstract:** A Quality by Design (QbD) approach was employed to develop and optimize a novel and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Carvedilol (CAR) and Ivabradine Hydrochloride (IVA) in pharmaceutical dosage form. The optimized method parameters were determined using a Central Composite Design (CCD) and included a run time of 8 minutes, flow rate of 1ml/min, and buffer composition of 60. The mobile phase comprised acetate buffer and acetonitrile (60:40) with detection at 289 nm. Retention times of 3.28 minutes for CAR and 2.12 minutes for IVA were achieved, ensuring efficient separation with high resolution and minimal tailing. Method validation confirmed precision, accuracy, linearity, robustness, and system suitability in compliance with ICH guidelines, establishing the method's reliability for routine use. The results indicate that the method is linear in the range of 5-30 µg/mL, with LOD and LOQ values for Carvedilol 2.16 µg/mL and 6.55 µg/mL, and for Ivabradine Hydrochloride 1.64 µg/mL and 4.97 µg/mL, highly accurate (98-102%), Precise (<2%), and robust (<2%). Green analytical chemistry assessment using GAPI, AGREE, and BAGI tools highlighted the method's environmental sustainability, practicality, and safety. The development of this method supports efficient quality control by providing a precise, eco-friendly analytical solution for simultaneous drug analysis. Additionally, its adaptability for future pharmaceutical applications makes it a valuable tool for enhancing process efficiency and meeting sustainability goals.

**Keywords:** Carvedilol; ivabradine hydrochloride; quality by design (QbD); high-performance liquid chromatography (HPLC); greenness.



## 1. Introduction

Carvedilol (CAR), chemically known as (±)-1-(Carbazol-4-yloxy)-3-{[2-(o-methoxyphenoxy)ethyl]amino}-2-propanol (Figure 1), is a non-selective beta-adrenergic antagonist with additional alpha-1 adrenergic blocking properties. This dual mechanism leads to vasodilation and a reduction in peripheral vascular resistance, effectively lowering blood pressure without inducing reflex tachycardia. Carvedilol's unique pharmacological profile also includes antioxidant, anti-inflammatory, and antiapoptotic properties, contributing to its

efficacy in treating conditions such as hypertension, heart failure, and ischemic heart disease [1,2].

Ivabradine (IVA), chemically designated as 3-(3-{[(7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl]methylamino}propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one hydrochloride (Figure 2), is a selective inhibitor of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, specifically targeting the funny current (If) in the sinoatrial node. By inhibiting this current, ivabradine prolongs diastolic depolarization, thereby reducing heart rate without affecting myocardial contractility or intracardiac conduction. This mechanism makes ivabradine particularly beneficial in managing chronic heart failure with reduced ejection fraction, as it effectively lowers heart rate and improves clinical outcomes [3,4].

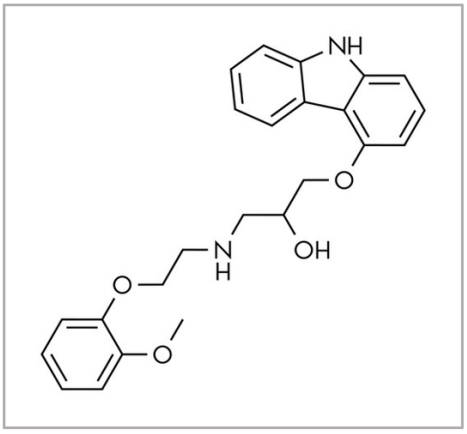



**Figure 1.** Structure of Carvedilol.

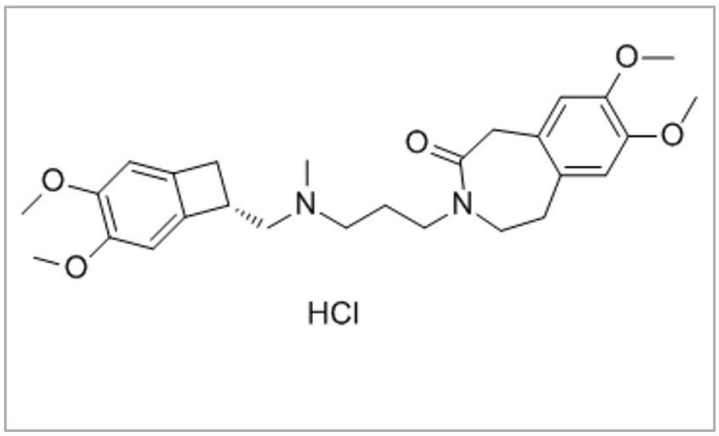



**Figure 2.** Structure of Ivabradine hydrochloride.

Various HPLC techniques have been reported in the literature for the analysis of Carvedilol and Ivabradine in combination, including stability-indicating RP-HPLC methods and simultaneous estimation methods in synthetic mixtures. These methods use different mobile phases and chromatographic conditions to achieve precise, accurate separation of the two drugs. However, a QbD-based RP-HPLC method for their simultaneous estimation has not been reported.

The application of Quality by Design (QbD) principles ensures robustness and method optimization by systematically identifying critical parameters such as mobile phase composition, flow rate, pH, column temperature, and detection wavelength. The combination of Carvedilol and Ivabradine is clinically significant in cardiovascular therapy, requiring precise, validated analytical methods to ensure quality and efficacy. Implementing Design of

Experiments (DoE) within a QbD framework enhances method understanding and robustness, offering advantages over traditional trial-and-error approaches [5].

The International Council for Harmonization (ICH) defines Quality by Design (QbD) as a structured development approach that starts with well-defined objectives and prioritizes a thorough understanding and control of both product and process, guided by scientific principles and quality risk management [6]. Analytical QbD (AQbD) extends these concepts to the analytical domain, enabling the creation of robust, efficient, and cost-effective methods. It focuses on determining critical method parameters and establishing a method-operable design region (MODR) by evaluating various influencing factors [7]. Experimental designs such as the BoxBehnken design (BBD) and central composite design (CCD) are commonly used in AQbD-driven pharmaceutical analysis. This study employs CCD due to its efficiency, as it requires fewer experimental runs while maintaining statistical significance and effectively linking input variables to responses [7]. The core objective of AQbD is to identify potential failure points, enhance method robustness, and define a design space that ensures compliance with system suitability requirements, thereby supporting ongoing life cycle management [8]. This research aims to develop and optimize a QbD-based RP-HPLC method for the simultaneous estimation of Carvedilol and Ivabradine hydrochloride in pharmaceutical formulations.

## 2. Materials and Methods

Reference standards, Carvedilol and ivabradine hydrochloride, were obtained from a reputable pharmaceutical company as gift samples. HPLC-grade methanol was procured from Aceto Pharma (India) PVT. LTD. Triple-distilled water was obtained from the Research Lab of Shri. D. D. Vispute College of Pharmacy and Research Center.

### *2.1. Instrument and chromatographic conditions.*

The chromatographic analysis of the active pharmaceutical ingredients (APIs) was performed using Agilent Technologies 1260 Infinity II, which included an autosampler and PDA detector in addition to Open Lab (Ezchrome) software for data processing. The Eclipse Plus C18 (4.6×150 mm, 3.5µm) analytical column was used for optimal separation. Detection was performed at 289 nm, which was found suitable for both Carvedilol and Ivabradine Hydrochloride based on their maximum absorbance in the UV spectra. The best conditions for drug separation were investigated using a range of solvents with different properties. Isocratic elution was used in the separation. The mobile phase was composed of acetate buffer and acetonitrile in 60:40% v/v ratio, at a flow rate of 1ml/min. The analysis was completed within an 8-minute run time, with the column maintained at 25°C. The injection volume for the method was set at 10µL. The mobile phase comprising acetate buffer (pH 2.8) and acetonitrile in a 60:40 v/v ratio was selected based on multiple method development trials. Acetate buffer was chosen for its buffering capacity near the target pH, which ensures good peak symmetry for both analytes. Acetonitrile was preferred over methanol for its lower viscosity, better elution strength, and improved peak sharpness. The ratio was optimized to achieve shorter retention times with adequate resolution and theoretical plates during robustness and method optimization trials.

*2.2. Preparation of standard solution and working solution.*

10 mg of carvedilol and 10 mg of ivabradine hydrochloride (Reference standards) were dissolved in 50 % methanol and made up to a volume of 10 ml with distilled water to achieve a concentration of 1000 µg/ml. The same solvent system was used to prepare further appropriate dilutions.

*2.3. Selection of detection wavelength.a*

Standard solutions of both drugs were analyzed using a UV spectrophotometer in spectrum mode over 200-400 nm, with methanol and water (50:50) as the reference solvent. The two drugs, CAR and IVA, exhibited their respective λmax at 285nm and 286nm. A detection wavelength of 289nm was chosen based on the overlain UV spectra, where both drugs showed maximum response.

*2.4. HPLC method development by QbD approach.*

The development of the HPLC method was carried out using an Analytical Quality by Design (AQbD) approach, as shown below.

*2.5. Selection of quality target product profile (QTPP).*

The Quality Target Product Profile (QTPP) defines the essential performance characteristics required to achieve accurate, reliable, and reproducible results. For the suggested HPLC technique, the retention time, theoretical plates, and peak symmetry were found as essential QTPP parameters [9].

*2.6. Determine critical quality attributes (CQA).*

Critical Quality Attributes (CQA) are factors that have a substantial impact on the method's performance and the accuracy of the findings. For this approach, run duration, flow rate, and Buffer concentration were determined as key factors that must be carefully regulated to keep the QTPP within an acceptable response range.

*2.7. Factorial design.*

The experimental design was based on the central composite response surface approach. The DesignExpert software® (Version 8.0.7.1) was used to analyze the results of the experiments in order to optimize the chromatographic settings. To choose acceptable chromatographic settings. To choose acceptable chromatographic conditions, run length (6,8 and 10 minutes), flow rate (0.8,1 and 1.2 mL/min), and buffer composition (55, 60, and 65) were chosen as independent variables (Table 1). While retention time, theoretical plates, and peak symmetry were chosen as dependent parameters for optimum separation efficiency. The experimental design matrix was constructed for the three specified variables at three distinct points using about 20 experimental runs.

**Table 1.** Coded value for independent variables.

| Factor | Coded values given factor | Levels | | |
|---|---|---|---|---|
| | | -1 | 0 | +1 |
| Run time | A | 6 | 8 | 10 |
| Flow rate | B | 0.8 | 1.0 | 1.2 |
| Buffer composition | C | 55 | 60 | 65 |

*2.8. Evaluation of experimental results and selection of final method condition.*

The CCD techniques were used to evaluate the method conditions. In the initial stage, variables including retention time, theoretical plates, and peak symmetry were evaluated. This approach discovered different chromatographic conditions for abiraterone acetate and prednisolone. Proven acceptable limits were established within robust zones, where deliberate alterations to method parameters had no effect on method quality, ensuring reliability throughout validation testing. If the experimental models did not yield the expected responses, parameter levels were adjusted and re-evaluated until satisfactory results were obtained [9]. The most suitable chromatographic conditions were optimized with Design Expert tools.

*2.9. Risk assessment.*

The final optimized method was chosen based on key attributes, ensuring that it is efficient and functional throughout the product's lifespan. To analyze the method's robustness and ruggedness, a risk-based approach was used, guided by the Quality by Design (QbD) principles defined in ICH Q8 and ICH Q9 [9].

*2.10. Analytical method validation.*

The goal of validating an analytical technique is to establish that it is suitable for its intended purpose. The designed HPLC technique for measuring carvedilol and ivabradine hydrochloride was validated using ICH Q2 (R1) guidelines [10].

*2.11. Linearity.*

Linearity was evaluated by diluting the standard stock solution to create aliquots with final concentrations of CAR and IVA ranging from 5-30 µg/mL. The column was injected with 20 µL of each mixture. A calibration curve was created by plotting peak area against drug concentration, and the regression equation and correlation coefficient were calculated.

*2.12. Precision.*

Precision refers to the method's capacity to analyze multiple replicates under varying conditions consistently. To determine precision, samples of both analytes (CAR and IVA) underwent interday and intraday quality control (QC)analysis. The acceptance criterion for %RSD was set at ≤2%.

*2.13. Accuracy.*

The method's accuracy was assessed using recovery studies on a marketed formulation at three levels: 80%, 100%, and 120% of the standard solution. The percentage recovery of CAR and IVA was computed, with an acceptability criterion of 98% to 102% based on ICH guidelines.

*2.14. LOD and LOQ.*

The limit of detection (LOD) represents the lowest concentration on the calibration curve with a signal-to-noise ratio (S/N) of ≥3. The limit of quantification (LOQ) is defined as the concentration yielding an S/N ratio of ≥10, with a %RSD (n=3) of ≤10%. The LOD was calculated by determining the lowest concentration that could be reliably distinguished from

the blank, assuring detection feasibility. The LOD and LOQ were determined using equations 1 and 2 according to ICH guidelines:

$$LOD = 3.3 \times \sigma \div SD \quad (1)$$

$$LOQ = 10 \times \sigma \div SD \quad (2)$$

σ = standard deviation

SD = slope

*2.15. Robustness.*

The influence of minor chromatographic modifications on retention time (RT) and peak area was used to measure robustness. Samples were analyzed using altered flow rates (0.8, 1.0, and 1.2 mL/min), detection wavelengths (287, 289, and 291 nm), and column oven temperature (20°C, 25°C, and 30°C). The effects of various technique adjustments were investigated, and the findings were used to establish the method's stability under minor changes.

*2.16. System suitability.*

To examine system performance, six duplicate samples of the CAR and IVA solutions were injected. The parameters tested were retention time, column efficiency, peak asymmetry, theoretical plates, and peak area. These parameters were utilized to guarantee that the system was reliable and consistent throughout the analysis.

*2.17. Assay.*

Before being pulverized, twenty pills were individually weighed to determine their average weight. The tablet powder, equivalent to 10 mg of CAR and 20 mg of IVA, was transferred to a 100 mL volumetric flask. To dissolve the powder, 20 mL of mobile phase was added to the mixture, which was then sonicated at a controlled temperature for 5 minutes. The volume was added to the mark using the same solvent. From this solution, 1.0 mL was pipetted into a 10 mL volumetric flask and diluted with mobile phase to the mark, then thoroughly mixed. The solution was filtered using a 0.45 µm membrane filter. Further dilutions were made using methanol and water (50:50) to achieve a final concentration of 20 µg/mL, which lies within the linearity range. The drug content in each tablet and the bulk drug were determined using a standard calibration curve.

*2.18. Green assessment.*

The growing demand to adopt sustainable processes to meet the Green Analytical Chemistry (GAC) criteria poses a significant challenge for the pharmaceutical sector. High-performance liquid chromatography (HPLC), one of the most widely used procedures across various phases of pharmaceutical analysis, generates substantial amounts of organic toxic waste. As a result, implementing GAC principles in pharmaceutical analysis has become essential. These principles can be summarized into four main areas: (1) eliminating or reducing reagent consumption in analytical procedures, (2) minimizing energy consumption, (3) managing analytical waste properly, and (4) enhancing operator safety [11].

The BAGI (Blue Analytical Greenness Index) tool complements existing green assessment tools like GAPI, Complex GAPI, AGREE, and AGREEprep. Unlike its green counterparts, BAGI focuses on the "blue" principles of White Analytical Chemistry (WAC), emphasizing practical aspects of analytical methods. This tool assesses ten key characteristics,

including the type of analysis, the number of analytes that are simultaneously determined, the number of samples that can be analyzed per hour, the type of reagents and materials in the analytical method, the required instrumentation, the number of samples that can be simultaneously treated, the requirement for preconcentration, the automation degree, the type of sample preparation, and the amount of sample. These criteria are used to generate a pictogram and a score that reflect the method's applicability and functionality. A sequential blue color scale is used to represent the final score, with dark blue indicating high compliance, followed by blue (medium), light blue (low), and white (no compliance). For an analytical method to be deemed "practical", a total score of 60 or higher is recommended [12].

## 3. Results and Discussion

During the initial phase of development, using a 50:50 v/v water-acetonitrile mixture as the mobile phase failed to produce a detectable peak. Subsequent trials with a methanol and water mixture in a 50:50 v/v ratio also showed no peak. Switching to an acetate buffer and acetonitrile mixture in a 50:50 v/v ratio resulted in flat peaks. Further adjustments to improve peak shape and symmetry lead to the optimized mobile phase composition of an acetate buffer to acetonitrile mixture in a 60:40 v/v ratio. The central composite design was then employed to optimize various parameters within the design space for better performance.

### *3.1. HPLC method development by QbD approach.*

#### 3.1.1. Quality target product profile (QTPP).

The selected Quality Target Product Profile (QTPP) parameters for optimizing HPLC chromatographic conditions included retention time, theoretical plates, and peak asymmetry for both drugs.

#### 3.1.2. Critical quality attributes.

Run time, Flow rate, and column temperature were identified as critical quality attributes (CQA) essential for ensuring the method's performance and reliability.

#### 3.1.3. Factorial design.

A Central Composite Design (CCD) was employed in HPLC method development to optimize the chromatographic conditions by evaluating the influence of three independent variables: run time, flow rate, and buffer composition. The design consisted of 20 experimental runs, and six responses were studied: retention times, number of theoretical plates (NTP), and peak symmetry for both Carvedilol and Ivabradine. The model was statistically evaluated, and the results are summarized in Table 2, which outlines the optimized design matrix and corresponding response values. These data helped in identifying the optimal conditions for the simultaneous estimation of both analytes.

**Table 2.** Optimization of parameters for analysis of carvedilol and ivabradine hydrochloride.

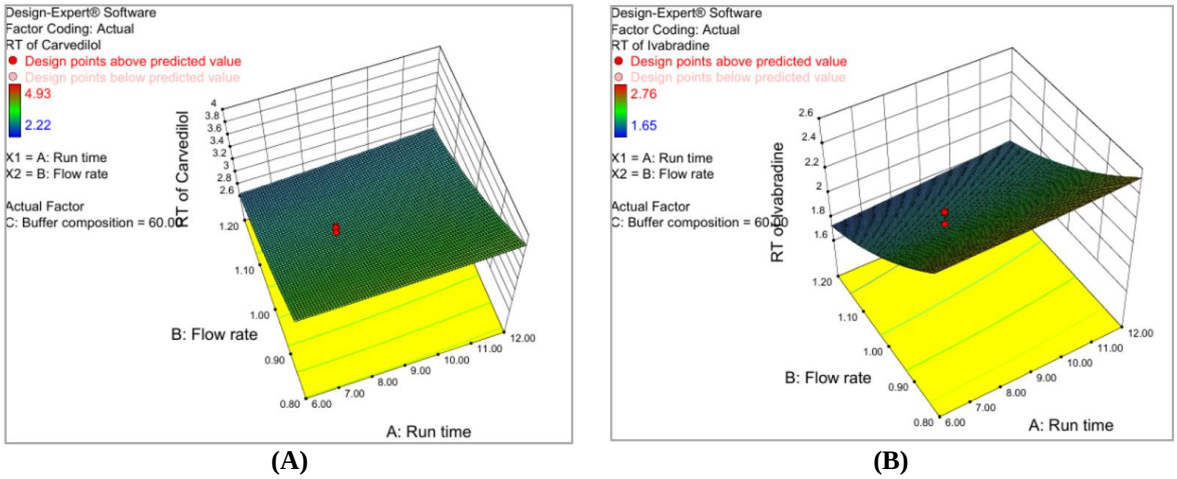
| Run | Factor 1 Run time | Factor 2: Flow rate | Factor 3 Buffer composition | Response 1 RT of Carvedilol | Response 2 RT of Ivabradine hydrochloride | Response 3 NTP of Carvedilol | Response 4 NTP of Ivabradine hydrochloride | Response 5: Peak symmetry of Carvedilol | Response 6 Peak symmetry of Ivabradine hydrochloride |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.00 | 1.00 | 60.00 | 3.16 | 2.07 | 2791 | 2861 | 1.05 | 0.96 |
| 2 | 6.00 | 0.80 | 55.00 | 3.3 | 2.45 | 3910 | 4035 | 1.03 | 1.19 |

| Run | Factor 1 Run time | Factor 2: Flow rate | Factor 3 Buffer composition | Response 1 RT of Carvedilol | Response 2 RT of Ivabradine hydrochloride | Response 3 NTP of Carvedilol | Response 4 NTP of Ivabradine hydrochloride | Response 5: Peak symmetry of Carvedilol | Response 6 Peak symmetry of Ivabradine hydrochloride |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 8.00 | 1.00 | 60.00 | 3.01 | 1.94 | 2764 | 2384 | 1.08 | 0.95 |
| 4 | 8.00 | 1.00 | 60.00 | 3.16 | 2.07 | 2881 | 2860 | 1.06 | 0.94 |
| 5 | 6.00 | 1.20 | 55.00 | 2.23 | 1.65 | 2436 | 2007 | 0.99 | 0.88 |
| 6 | 8.00 | 1.00 | 60.00 | 2.99 | 1.95 | 2717 | 2422 | 1.05 | 0.95 |
| 7 | 6.00 | 1.20 | 65.00 | 3.43 | 1.89 | 1638 | 1169 | 1.01 | 0.98 |
| 8 | 8.00 | 1.00 | 60.00 | 3.27 | 2.17 | 1886 | 2155 | 1.04 | 0.87 |
| 9 | 10.00 | 0.80 | 55.00 | 3.29 | 2.45 | 2949 | 2744 | 1.16 | 0.99 |
| 10 | 4.64 | 1.00 | 60.00 | 3 | 1.95 | 2305 | 2000 | 1.04 | 0.88 |
| 11 | 11.36 | 1.00 | 60.00 | 3.15 | 2.07 | 2267 | 2207 | 1.08 | 0.95 |
| 12 | 10.00 | 1.20 | 55.00 | 2.22 | 1.65 | 1888 | 1815 | 1.02 | 0.88 |
| 13 | 8.00 | 1.00 | 68.41 | 4.69 | 2.17 | 1296 | 1007 | 1.13 | 1 |
| 14 | 8.00 | 1.34 | 60.00 | 2.51 | 1.67 | 1319 | 1459 | 0.99 | 0.82 |
| 15 | 10.00 | 0.80 | 65.00 | 4.89 | 2.76 | 2278 | 2229 | 1.15 | 0.94 |
| 16 | 10.00 | 1.20 | 65.00 | 3.53 | 1.99 | 1450 | 1292 | 1.06 | 1.18 |
| 17 | 8.00 | 1.00 | 51.59 | 2.47 | 1.97 | 2333 | 2936 | 0.98 | 0.79 |
| 18 | 8.00 | 0.66 | 60.00 | 4.19 | 2.75 | 3665 | 2727 | 1.38 | 1.07 |
| 19 | 6.00 | 0.80 | 65.00 | 4.93 | 2.76 | 2320 | 2369 | 1.17 | 0.93 |
| 20 | 8.00 | 1.00 | 60.00 | 3.25 | 2.17 | 1913 | 2060 | 1.004 | 0.9 |

3.1.4. Design space.

The response surface study type, CCD, was employed for 20 runs. The suggested CCD experimental design was used, and run time (A), flow rate (B), and buffer composition (C) were measured against six responses: retention time. NTP, and Peak symmetry for both Carvedilol(CAR) and Ivabradine Hydrochloride(IVA).

The response surface and contour plots in Figure 3A and 3B were interpreted better to understand the influence of method variables on retention times. Decreasing the flow rate and the buffer composition increased retention time for both Carvedilol and Ivabradine, indicating an inverse relationship. Similarly, shorter run times also caused a slight increase in retention, as expected. These trends support selecting optimized conditions that balance analysis time and resolution efficiency.




**(A)** **(B)**

**Figure 3.** 3D surface plot for the effect of the combination of factors on **(A)** retention time of Carvedilol; **(B)** retention time of ivabradine hydrochloride by using central composite design.

From Figure 3(A) and the equation for Retention Time (RT) of Carvedilol in terms of actual values:

$$RT = +22.01188 - 0.11142\,A - 2.90606\,B - 0.65833\,C + 0.043750\,A\,B + 0.001000\,A\,C - 0.090000\,B\,C + 0.00114845\,A^2 + 2.54552\,B^2 + 0.00732553\,C^2 \quad \textbf{(3)}$$

From Equation 3, it was concluded that the $\beta_1$ negative coefficient (-0.11142) indicates that as the run time (A) decreases, the retention time of Carvedilol increases. The $\beta_2$ negative coefficient (-2.90606) suggests that as the flow rate (B) decreases, the retention time increases significantly, demonstrating an inverse relationship. The $\beta_3$ negative coefficient (-0.65833) indicates that as the buffer composition (C) decreases, the retention time increases.

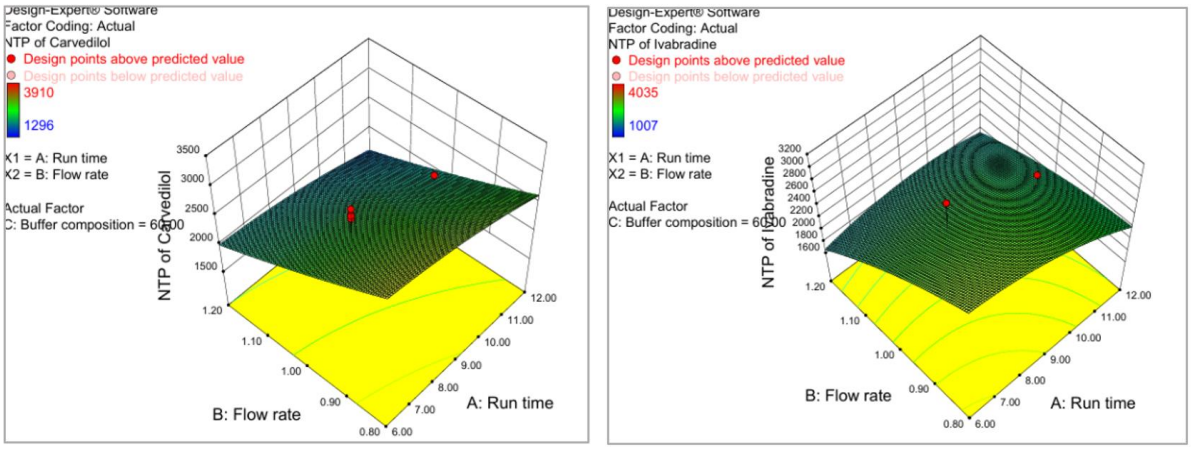
From Figure 3(B) and the equation for Retention Time (RT) of Ivabradine Hydrochloride in terms of actual values:

$$RT = +8.41552 - 0.10756\,A - 5.49129\,B - 0.099160\,C + 0.031250\,A\,B + 0.001250\,A\,C - 0.005000\,B\,C + 0.000772717\,A^2 + 1.84504\,B^2 + 0.000972163\,C^2 \quad \textbf{(4)}$$

From Equation 4, it was concluded that the $\beta_1$ negative coefficient (-0.10756) indicates that as the run time (A) decreases, the retention time of Ivabradine Hydrochloride increases. The $\beta_2$ negative coefficient (-5.49129) suggests that as the flow rate (B) decreases, the retention time increases significantly, demonstrating an inverse relationship. The $\beta_3$ negative coefficient (-0.099160) indicates that as the buffer composition (C) decreases, the retention time increases.

A Similar pattern was seen for both drugs, confirming that flow rate and buffer composition are the most influential parameters affecting retention.

Figures 4A and 4B depict the effect of method variables on the number of theoretical plates (NTP). Decreasing flow rate significantly improved column efficiency for both Carvedilol and Ivabradine, likely due to enhanced mass transfer and longer residence time. Higher buffer composition also contributed positively to efficiency, while shorter run times showed a minor yet supportive effect.




**(A)** **(B)**

**Figure 4.** 3D surface plot for the effect of the combination of factors on **(A)** NTP of Carvedilol; **(B)** NTP of ivabradine hydrochloride by using central composite design.

From Figure 4(A) and the equation for Number of Theoretical Plates (NTP) of Carvedilol in terms of actual values:

$$NTP = -2096.42592 - 968.45125\,A - 13169.00266\,B + 635.67652\,C + 83.43750\,A\,B + 15.98750\,A\,C + 128.12500\,B\,C - 8.76523\,A^2 + 944.27686\,B^2 - 8.07045\,C^2 \quad \textbf{(5)}$$

From equation 5, it was concluded that the $\beta_1$ negative coefficient (-968.45125) indicates that as the run time (A) decreases, the number of theoretical plates of Carvedilol increases. The $\beta_2$ negative coefficient (-13169.00266) suggests that as the flow rate (B)

decreases, the number of theoretical plates increases significantly, demonstrating an inverse relationship. The $\beta_3$ positive coefficient (+635.67652) indicates that as the buffer composition (C) increases, the number of theoretical plates also increases.

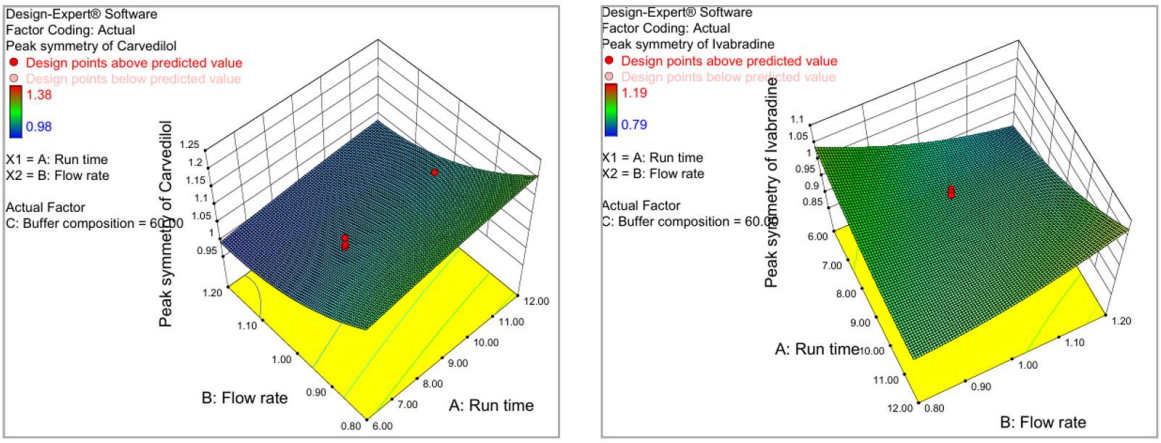
From Figure 4(B) and the equation for Number of Theoretical Plates (NTP) of Ivabradine Hydrochloride in terms of actual values:

$$NTP = +6677.76268 - 1215.78532\,A - 7621.23663\,B + 297.34395\,C + 425.62500\,A\,B + 18.32500\,A\,C + 102.50000\,B\,C - 21.96947\,A^2 - 2289.75465\,B^2 - 5.38188\,C^2 \quad \textbf{(6)}$$

From equation 6, It was concluded that the $\beta_1$ negative coefficient (-1215.78532) indicates that as the run time (A) decreases, the number of theoretical plates of Ivabradine Hydrochloride increases. The $\beta_2$ negative coefficient (-7621.23663) suggests that as the flow rate (B) decreases, the number of theoretical plates increases significantly, demonstrating an inverse relationship. The $\beta_3$ positive coefficient (+297.34395) indicates that as the buffer composition (C) increases, the number of theoretical plates also increases.

The equation for NTP of CAR indicated that flow rate ($\beta_2$ = –13169.00) had the strongest influence. For IVA, a similar trend was observed with a slightly lesser magnitude.

Figures 5A and 5B represent the impact of parameters on peak symmetry. For Carvedilol, lower flow rates and higher buffer composition improved symmetry, suggesting better peak shape under gentler elution conditions. In contrast, Ivabradine showed improved symmetry at lower flow rate and buffer content, reflecting differences in molecular interactions and polarity.




**(A)** **(B)**

**Figure 5.** 3D surface plot for the effect of the combination of factors on **(A)** peak symmetry of Carvedilol; **(B)** peak symmetry of ivabradine hydrochloride by using central composite design.

From Figure 5(A) and the equation for Peak Symmetry of Carvedilol in terms of actual values:

$$Peak\ Symmetry = +0.13117 + 0.12323\,A - 1.92051\,B + 0.045041\,C - 0.009375\,A\,B - 0.001625\,A\,C - 0.008750\,B\,C - 0.000433803\,A^2 + 1.06147\,B^2 - 0.000140119\,C^2 \quad \textbf{(7)}$$

From equation 7, it was concluded that the $\beta_1$ positive coefficient (+0.12323) indicates that as the run time (A) increases, the peak symmetry of Carvedilol also increases. The $\beta_2$

negative coefficient (-1.92051) suggests that as the flow rate (B) decreases, the peak symmetry increases significantly, demonstrating an inverse relationship. The $\beta_3$ positive coefficient (+0.045041) indicates that as the buffer composition (C) increases, the peak symmetry also increases.

From Figure 5(B) and the equation for Peak Symmetry of Ivabradine Hydrochloride in terms of actual values:

$$Peak\ Symmetry = +10.90084 - 0.47212\ A - 7.62467\ B - 0.14623\ C + 0.12187\ A\ B + 0.005125\ A\ C + 0.088750\ B\ C + 0.00296406\ A^2 + 0.56157\ B^2 + 0.000191407\ C^2 \quad \textbf{(8)}$$

From equation 8, it was concluded that the $\beta_1$ negative coefficient (-0.47212) indicates that as the run time (A) decreases, the peak symmetry of Ivabradine Hydrochloride increases. The $\beta_2$ negative coefficient (-7.62467) suggests that as the flow rate (B) decreases, the peak symmetry increases significantly, demonstrating an inverse relationship. The $\beta_3$ negative coefficient (-0.14623) indicates that as the buffer composition (C) decreases, the peak symmetry also increases.

These optimization results, combined with response equations, clearly demonstrate the mechanistic influence of each parameter and support the robustness of the final selected method.

3.1.5. Optimized condition obtained.

The ideal method was found by numerical optimization by "trading off" various CAAs in order to achieve the desired objectives, i.e., maximization of theoretical plates and minimization of retention time, resulting in a desirability function close to 1. The optimized conditions revealed that a run time of 8 minutes, a flow rate of 1 mL/min, and a column temperature of 25°C resulted in desirability near 1.0 and all CAAs within the intended limits. Table 3 shows the optimized run time, flow rate, and column temperature parameters, as well as the projected responses. Optimized chromatographic conditions are explained in Table 4.

The response surface and contour plots highlighted how method variables influenced retention times. Decreasing the flow rate and buffer composition increased retention for both Carvedilol and Ivabradine, while shorter run times slightly extended elution. These trends are not only statistically significant but also chemically justified. Lower flow rates increase the analyte's interaction time with the stationary phase, thereby delaying elution. Similarly, a higher aqueous buffer composition increases the polarity of the mobile phase, thereby enhancing the retention of moderately lipophilic analytes such as Carvedilol. The observed difference in Ivabradine's retention is attributed to its greater polarity, which causes it to elute faster under similar conditions. Furthermore, changes in buffer composition and pH can influence ionization, thereby impacting solubility and interactions with the stationary phase, which explains variations in peak shape and retention behavior.

**Table 3.** Optimized solution for method development.

| Run time (min) | Flow rate (ml/min) | Temp. | Buffer composition | Retention time of Carvedilol | Retention time of ivabradine hydrochloride | NTP of Carvedilol | NTP of ivabradine hydrochloride | The peak symmetry of Carvedilol | The peak symmetry of ivabradine hydrochloride |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 1 | 25°C | 60 | 3.2 min | 2.1 min | 2369 | 2397 | 0.9 | 1.0 |

**Table 4.** Optimized chromatographic conditions.

| Chromatographic conditions | Optimized chromatographic conditions |
|---|---|
| Column | Eclipse Plus C18 (4.6×150mm,3.5µm) |
| Wavelength | 289 nm (Iso absorptive Point) |
| Column Temperature | 25°C |
| Flow Rate | 1mL/min |
| Injection Volume | 20µL |
| Run Time | 8 min |
| Mobile Phase | Acetate Buffer: ACN (60:40) v/v [pH 2.8] |
| Diluent | Water: methanol (50:50) |

*3.2. Method validation.*

3.2.1. System suitability.

The system suitability test was performed on a representative chromatogram to evaluate parameters, including retention time: 3.2 min for CAR and 2.1 min for IVA. The theoretical plates for CAR were estimated at 2369, and for IVA at 2397, whereas the tailing factors for CAR were 0.9 and for IVA 1.0. Figure 6 represents the 3D surface plot of the desirability function used to determine the optimal chromatographic conditions. The plot combines all response goals—minimum retention time, maximum theoretical plates, and ideal peak symmetry—into a single composite score. A desirability value close to 1.0 indicates that all targeted responses are simultaneously satisfied. The optimized conditions obtained (flow rate: 1.0 mL/min, buffer composition: 60%, and run time: 8 min) corresponded to a high overall desirability, confirming the robustness and suitability of the method.

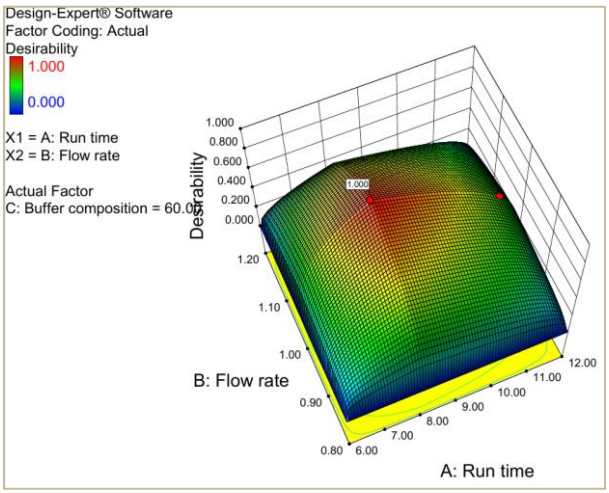



**Figure 6.** 3D surface plot showing composite desirability based on method optimization using CCD.3.2.2 Peak identification and UV spectral analysis.

Peak identity was confirmed using retention time and UV spectral analysis through the UV detector. Standard solutions of Carvedilol and Ivabradine Hydrochloride were scanned between 200–400 nm, and both exhibited a sharp λmax at 289 nm. This wavelength was selected for detection to ensure simultaneous sensitivity for both drugs. Overlay of spectra also confirmed the absence of major interfering peaks, supporting the selectivity of the method. The UV spectra of both drugs are shown in Figures 7 and 8.

This wavelength was selected for quantitative analysis due to adequate absorbance intensity for both analytes. In the chromatograms obtained for Carvedilol, two additional peaks (Peak 4 and Peak 5) were observed. These were identified as related impurities of Carvedilol, consistent with previous reports by Nguyen *et al.*, where similar peaks were reported during

the simultaneous determination of Carvedilol and its five related impurities using capillary electrophoresis [13]. These impurity peaks did not interfere with the main analyte peaks and remained well-resolved, confirming the selectivity of the developed method.

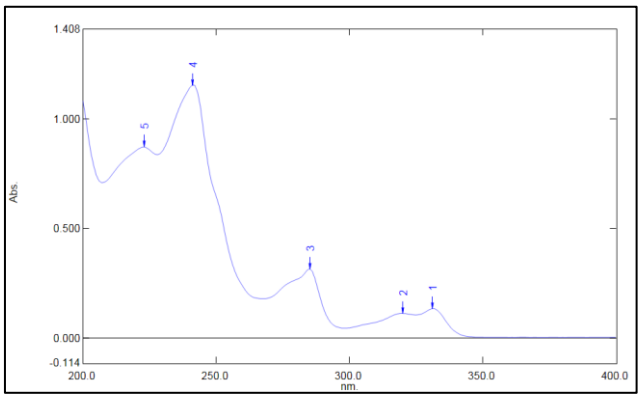



**Figure 7.** UV absorbance spectrum of Carvedilol showing maximum absorbance at 285 nm (Peak 3).

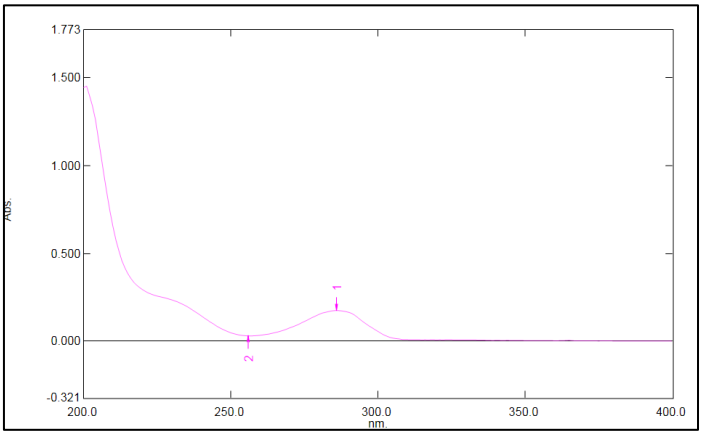



**Figure 8.** UV absorbance spectrum of Ivabradine, showing maximum absorbance at 286 nm (Peak 1)

3.2.3. Linearity.

Calibration curves were constructed for Carvedilol (CAR) and Ivabradine Hydrochloride (IVA) over the concentration range of 5–30 µg/mL, as shown in Figure 9. The relationship between peak area and concentration was evaluated using linear least squares regression. The resulting regression equations were equations 9 and 10:

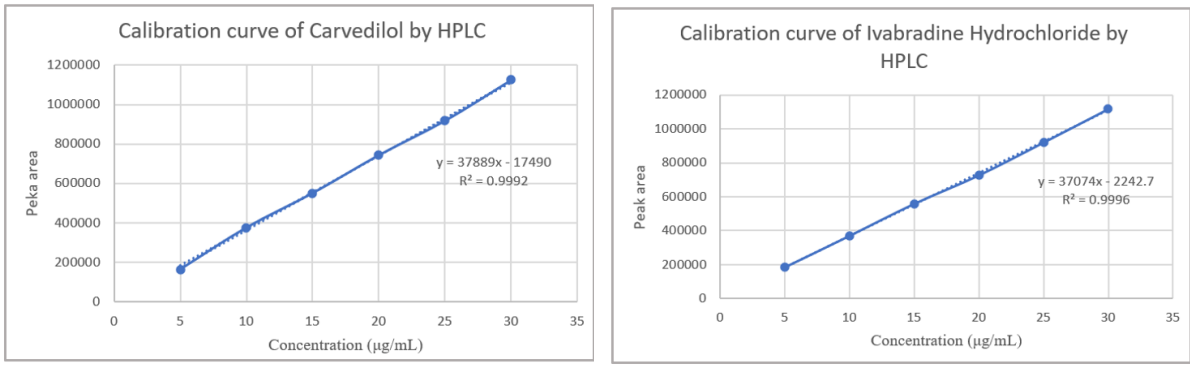
$$CAR: y = 37889x + 17490\ (R^2 = 0.9992) \quad \textbf{(9)}$$

$$IVA: y = 37074x + 2242.7\ (R^2 = 0.9996) \quad \textbf{(10)}$$

These values demonstrate excellent linearity for both drugs, as summarized in Table 5.

**Table 5.** Linearity of carvedilol and ivabradine hydrochloride.

| Sr no. | Carvedilol | | Ivabradine hydrochloride | |
|---|---|---|---|---|
| | Concentration | Peak area | Concentration | Peak area |
| 1 | 5 | 162710 | 5 | 184600 |
| 2 | 10 | 375395 | 10 | 369862 |
| 3 | 15 | 549794 | 15 | 559076 |
| 4 | 20 | 743118 | 20 | 726985 |
| 5 | 25 | 917442 | 25 | 920749 |
| 6 | 30 | 1124925 | 30 | 1118093 |




**(A)** **(B)**

**Figure 9.** Linearity graph of **(A)** Carvedilol; **(B)** Ivabradine hydrochloride.

3.2.4. Precision.

The precision of the method was evaluated through both interday and intraday analysis for Carvedilol and Ivabradine Hydrochloride. Six replicate injections were performed for each analyte at a concentration of 15 µg/mL. The %RSD values for peak area, number of theoretical plates (NTP), tailing factor, and retention time were all found to be below 2% for both drugs. These low %RSD values confirm that the method is highly precise, with minimal variability in the results, as detailed in Tables 6 and 7.

**Table 6.** Intraday and Interday precision data for Carvedilol.

| Sr.no | Concentration (ug/ml) | Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak area | Theoretical plates | Tailing factor | Retention time | Peak area | Theoretical plates | Tailing factor | Retention time |
| 1 | 15 | 567942 | 2317 | 1 | 3.28 | 568502 | 2320 | 1 | 3.28 |
| 2 | 15 | 557694 | 2329 | 1 | 3.29 | 557919 | 2325 | 1 | 3.28 |
| 3 | 15 | 552869 | 2317 | 1 | 3.29 | 553250 | 2315 | 1 | 3.29 |
| 4 | 15 | 564450 | 2279 | 1 | 3.29 | 564167 | 2285 | 1 | 3.29 |
| 5 | 15 | 564069 | 2258 | 1 | 3.29 | 563990 | 2260 | 1 | 3.29 |
| 6 | 15 | 564957 | 2258 | 1 | 3.29 | 564801 | 2255 | 1 | 3.29 |
| | Mean | 561996.833 | 2293 | 1 | 3.2883 | 562104.833 | 2293.3333 | 1 | 3.2866 |
| | Standard deviation | 5590.1916 | 31.9186 | 0 | 0.0040 | 5521.3264 | 31.0912 | 0 | 0.0051 |
| | %RSD | 0.9947 | 1.3920 | 0 | 0.1241 | 0.9806 | 1.3557 | 0 | 0.1571 |

**Table 7.** Intraday and Interday precision data for Ivabradine hydrochloride.

| Sr.no | Concentration (ug/ml) | Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak area | Theoretical plates | Tailing factor | Retention time | Peak area | Theoretical plates | Tailing factor | Retention time |
| 1 | 15 | 580554 | 2198 | 0.9 | 2.11 | 581235 | 2205 | 0.9 | 2.11 |
| 2 | 15 | 579163 | 2163 | 0.9 | 2.11 | 578929 | 2155 | 0.9 | 2.11 |
| 3 | 15 | 579415 | 2179 | 0.9 | 2.12 | 579622 | 2183 | 0.9 | 2.12 |
| 4 | 15 | 579425 | 2143 | 0.9 | 2.12 | 578745 | 2138 | 0.9 | 2.12 |
| 5 | 15 | 579330 | 2110 | 0.9 | 2.11 | 579103 | 2115 | 0.9 | 2.11 |
| 6 | 15 | 579452 | 2124 | 0.9 | 2.12 | 579311 | 2120 | 0.9 | 2.12 |
| | Mean | 579556.5 | 2152.8333 | 0.9 | 2.115 | 579490.8333 | 2152.6666 | 0.9 | 2.115 |
| | Standard deviation | 499.8770 | 33.4389 | 0 | 0.0054 | 907.08333 | 35.6800 | 0 | 0.0054 |
| | % RSD | 0.08 | 1.55 | 0 | 0.2589 | 0.1565 | 1.657 | 0 | 0.2589 |

3.2.5. Accuracy.

The accuracy of the optimized method was assessed by calculating the percentage recovery of Carvedilol and Ivabradine Hydrochloride at three concentration levels: 80%, 100%, and 120% of the nominal concentration, as illustrated in Figure 10. The recovery values for both analytes ranged from 98% to 102%, which falls within the acceptable limits specified by ICH guidelines. These results confirm that the method is accurate and reliable, as summarized in Tables 8 and 9.

**Table 8.** Accuracy data for Carvedilol.

| Level | Tablet powder solution added (ug/ml) | Standard stalk solution added (ug/ml | Total amount (ug/ml) | Peak area | Calculated concentration | %Recovery (%) | Standard deviation | %RSD |
|---|---|---|---|---|---|---|---|---|
| 80% | 15 | 12 | 27 | 1045800 | 27.140067 | 100.63 | 0.0377 | 0.1388 |
| | 15 | 12 | 27 | 1046366 | 27.1550054 | | | |
| | 15 | 12 | 27 | 1048509 | 27.2115654 | | | |
| 100% | 15 | 15 | 30 | 1164003 | 30.2597852 | 101.76 | 0.2322 | 0.7608 |
| | 15 | 15 | 30 | 1179845 | 30.6779012 | | | |
| | 15 | 15 | 30 | 1178564 | 30.644092 | | | |
| 120% | 15 | 18 | 33 | 1292679 | 33.655916 | 101.74 | 0.2020 | 0.6017 |
| | 15 | 18 | 33 | 1295294 | 33.7249334 | | | |
| | 15 | 18 | 33 | 1280922 | 33.3456148 | | | |

**Table 9.** Accuracy data for Ivabradine hydrochloride.

| Level | Tablet powder solution added (ug/ml) | Standard stalk solution added (ug/ml | Total amount (ug/ml) | Peak area | Calculated concentration | %Recovery (%) | Standard deviation | %RSD |
|---|---|---|---|---|---|---|---|---|
| 80% | 15 | 12 | 27 | 1010894 | 27.2064330 | 101.58 | 0.1975 | 0.7201 |
| | 15 | 12 | 27 | 1024983 | 27.5864568 | | | |
| | 15 | 12 | 27 | 1014477 | 27.3030776 | | | |
| 100% | 15 | 15 | 30 | 1128593 | 30.3811377 | 101.44 | 0.0352 | 0.1159 |
| | 15 | 15 | 30 | 1126366 | 30.3210686 | | | |
| | 15 | 15 | 30 | 1128667 | 30.3831337 | | | |
| 120% | 15 | 18 | 33 | 1227232 | 33.0417354 | 101.58 | 0.1161 | 0.3499 |
| | 15 | 18 | 33 | 1227600 | 33.0516615 | | | |
| | 15 | 18 | 33 | 1234869 | 33.2477288 | | | |

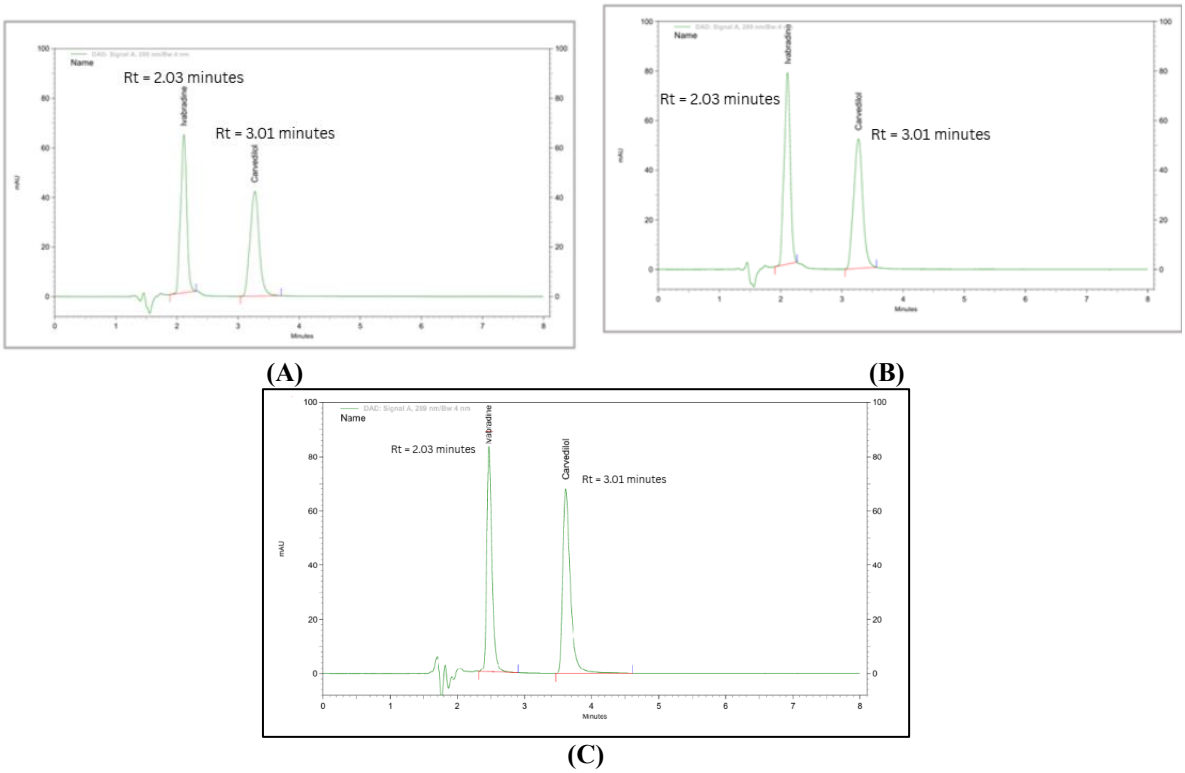



**Figure 10.** Chromatograms for accuracy **(A)** 80%; **(B)** 100%; **(C)** 120%.

3.2.6. Robustness.

The robustness of the HPLC method was evaluated by analyzing samples under deliberately varied chromatographic conditions. The flow rate was adjusted from the standard 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. Similarly, the detection wavelength was varied by ±2%, and the column temperature was modified by ±10°C. The impact of these changes on retention time and peak characteristics was assessed, with the results summarized in Tables 10 and 11.

**Table 10.** Robustness study of Carvedilol.

| **Flow rate** | **Flow minus (0.8 ml/min)** | | | **Flow plus (1.2 ml/min)** | | |
|---|---|---|---|---|---|---|
| **Sr no.** | **Peak area** | **Retention time** | **Theoretical plates** | **Peak area** | **Retention time** | **Theoretical plates** |
| 1 | 1093424 | 3.61 | 5708 | 739685 | 2.42 | 5103 |
| 2 | 1101258 | 3.61 | 5760 | 752198 | 2.42 | 5162 |
| 3 | 1087895 | 3.61 | 5682 | 734567 | 2.42 | 5087 |
| Mean | 1094192.33 | 3.61 | 5716.67 | 742150.00 | 2.42 | 5117.33 |
| Standard deviation | 6714.55 | 0 | 39.72 | 9070.29 | 0 | 39.50 |
| %RSD | 0.61 | 0 | 0.69 | 1.22 | 0 | 0.77 |
| Temperature | Temperature minus(20°C) | | | Temperature plus(30°C) | | |
| Sr no. | Peak area | Retention time | Theoretical plate | Peak area | Retention time | Theoretical plates |
| 1 | 888274 | 2.93 | 5046 | 885345 | 2.87 | 5296 |
| 2 | 902145 | 2.93 | 5123 | 898712 | 2.87 | 5380 |
| 3 | 880321 | 2.93 | 5009 | 879564 | 2.87 | 5264 |
| Mean | 890246.67 | 2.93 | 5059.33 | 888207 | 2.87 | 5313.33 |
| Standard deviation | 11044.92 | 0 | 58.16 | 10374.43 | 0 | 59.91 |
| %RSD | 1.24 | 0 | 1.15 | 1.17 | 0 | 1.13 |
| Wavelength | Wavelength minus (287 nm) | | | Wavelength plus(2nm) | | |
| Sr no. | Peak area | Retention time | Theoretical plate | Peak area | Retention time | Theoretical plate |
| 1 | 1264261 | 2.87 | 5321 | 541809 | 2.88 | 5066 |
| 2 | 1278943 | 2.87 | 5239 | 552123 | 2.88 | 5140 |
| 3 | 1256789 | 2.87 | 5207 | 534892 | 2.88 | 5028 |
| Mean | 1266664.33 | 2.87 | 5255.67 | 542921.33 | 2.88 | 5076.33 |
| Standard deviation | 11270.84 | 0 | 58.80 | 8699 | 0 | 59.18 |
| %RSD | 0.89 | 0 | 1.12 | 1.60 | 0 | 1.17 |

**Table 11.** Robustness study of Ivabradine hydrochloride.

| **Flow rate** | **Flow minus (0.8 ml/min)** | | | **Flow plus (1.2 ml/min)** | | |
|---|---|---|---|---|---|---|
| **Sr no.** | **Peak area** | **Retention time** | **Theoretical plates** | **Peak area** | **Retention time** | **Theoretical plates** |
| 1 | 900358 | 2.47 | 5662 | 514222 | 1.65 | 4977 |
| 2 | 912467 | 2.47 | 5721 | 521345 | 1.65 | 5032 |
| 3 | 895214 | 2.47 | 5634 | 509876 | 1.65 | 4951 |
| Mean | 902679.67 | 2.47 | 5672.33 | 515177.67 | 1.65 | 4986.67 |
| Standard deviation | 8668.30 | 0 | 43.79 | 5764.67 | 0 | 40.62 |
| %RSD | 0.96 | 0 | 0.77 | 1.12 | 0 | 0.81 |
| Temperature | Temperature minus (20°C) | | | Temperature plus (30°C) | | |
| Sr no. | Peak area | Retention time | Theoretical plate | Peak area | Retention time | Theoretical plates |
| 1 | 731643 | 1.99 | 4944 | 732480 | 1.97 | 4977 |
| 2 | 745321 | 1.99 | 5010 | 738912 | 1.97 | 5035 |
| 3 | 728956 | 1.99 | 4923 | 730214 | 1.97 | 4958 |
| Mean | 735306.67 | 1.99 | 4959 | 734168.67 | 1.97 | 4990 |
| Standard deviation | 8590.89 | 0 | 44.54 | 4912.39 | 0 | 38.44 |
| %RSD | 1.17 | 0 | 0.90 | 0.67 | 0 | 0.77 |
| Wavelength | Wavelength minus (287 nm) | | | Wavelength plus(2nm) | | |
| Sr no. | Peak area | Retention time | Theoretical plate | Peak area | Retention time | Theoretical plate |
| 1 | 748351 | 1.98 | 4927 | 688986 | 1.98 | 4863 |
| 2 | 759642 | 1.98 | 4995 | 698754 | 1.98 | 4931 |
| 3 | 742198 | 1.98 | 4901 | 683217 | 1.98 | 4815 |
| Mean | 750063.67 | 1.98 | 4941 | 689317 | 1.98 | 4869.67 |
| Standard deviation | 8765.49 | 0 | 47.68 | 6378.72 | 0 | 58.37 |
| %RSD | 1.17 | 0 | 0.97 | 0.92 | 0 | 1.20 |

3.2.7. Limit of detection and limit of quantification.

The limit of detection (LOD) was found to be 2.16 µg/mL for Carvedilol (CAR) and 1.64 µg/mL for Ivabradine Hydrochloride (IVA). The limit of quantification (LOQ) was determined to be 6.55 µg/mL for CAR and 4.97 µg/mL for IVA.

3.2.8. Assay.

When the optimized method was applied to tablet formulations, the chromatogram revealed retention times of 3.29 minutes for Carvedilol (CAR) and 2.11 minutes for Ivabradine Hydrochloride (IVA), as shown in Figure 11. The assay results showed drug purities of 98.83% for CAR and 98.86% for IVA, based on the labeled tablet claims. These findings confirm that the method can accurately detect and quantify both analytes in the presence of common tablet excipients.

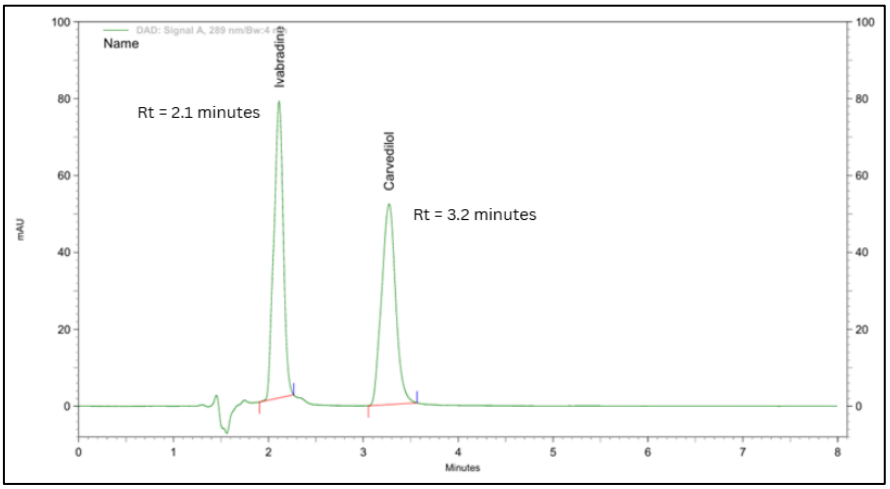



**Figure 11.** Optimized method chromatogram.

**Table 12.** Key validation data summary.

| Validation parameter | Carvedilol | Ivabradine hydrochloride | Acceptance criteria |
|---|---|---|---|
| Linearity Range (µg/ml) | 5-30 µg/mL | 5-30 µg/mL | $r^2 \geq 0.99$ |
| Accuracy (% Recovery) | 100.63 - 101.76 % | 101.44 – 101.58 % | 98 – 102 % |
| Intraday Precision (%RSD) | 0.9 | 0.08 | ≤2% |
| Interday Precision (%RSD) | 0.9 | 0.1 | ≤2% |
| LOD (µg/ml) | 2.16 µg/mL | 1.64 µg/mL | - |
| LOQ (µg/ml) | 6.55 µg/mL | 4.97 µg/mL | - |
| Robustness | No significant change | No significant change | System suitability maintained |
| Assay (% Purity) | 98.83 % | 98.86 % | 98-102 |

3.2.9. Greenness assessment.

The green analytical method's index assesses the feasibility of the various processes involved in the specified method. It takes into account sample preparation, handling, chemicals consumed, and instrumentation. The evaluation employs a color-coding scheme: green represents a minimal environmental impact, yellow indicates a moderate impact, and red highlights a significant impact, as shown in Figure 12. The HPLC technology employed demonstrated environmentally friendly attributes, with all parameters falling within the green zones, illustrated in Figure 11 [14,15]. Furthermore, the AGREE tool was utilized to determine the environmental friendliness profile of the analytical procedures using numerical values. The result obtained was 0.86, as shown in Figure 13, confirming the outstanding green attributes of the developed HPLC method. The assessment parameter is based on Green Analytical

Chemistry (GAC) principles and expressed on a 0-1 scale. Scores closer to 0 indicate a greater environmental risk and a more hazardous method, while scores closer to 1 indicate a greener method with minimal impact on both the system and the analyst [16].

Blue Applicability Grade Index (BAGI) evaluates ten main attributes of an analytical procedure in terms of practicality. The results of the proposed method performed using BAGI are presented in Figure 14. The assessed method obtained a high score (82.5), higher than 60, indicating its practicality [12].

To further justify the advantages of the developed method, a comparison was made with reported non-QbD HPLC methods for Carvedilol and Ivabradine Hydrochloride. Dey *et al*. developed a method for Carvedilol with a longer run time and high organic solvent consumption [17]. Rao *et al*. reported both HPLC and HPTLC methods using methanol and phosphate buffer with less greenness consideration [18]. Similarly, Seerapu and Srinivasan reported a method for Ivabradine with longer retention and the use of phosphate buffer [19]. In contrast, the present method was developed using the QbD approach and showed improved retention time, lower solvent consumption, and excellent greenness scores (GAPI, AGREE 0.86). These comparisons further confirm the superiority of the developed method in both environmental friendliness and robustness.

GAPI uses three color scales, which consist of yellow, red, and green to represent the ecological impact of each step of the analytical method. It gives the environmental impact level, where green signifies low impact, yellow denotes moderate impact, and red indicates high impact. The results show that it aligns with environmentally sustainable practices (Figure 12). These analytical approaches exhibited characteristics indicative of “green methods

Although the earlier version of the GAPI tool did not generate a detailed pictogram with colored scoring zones, the method was qualitatively assessed against GAPI's 15 criteria. The HPLC procedure required minimal sample preparation, used methanol and water as relatively safer solvents, and avoided derivatization or toxic reagents. The short run time and low solvent consumption further support its environmental compatibility. Based on these factors, the method can be classified as environmentally friendly in accordance with green analytical chemistry principles.

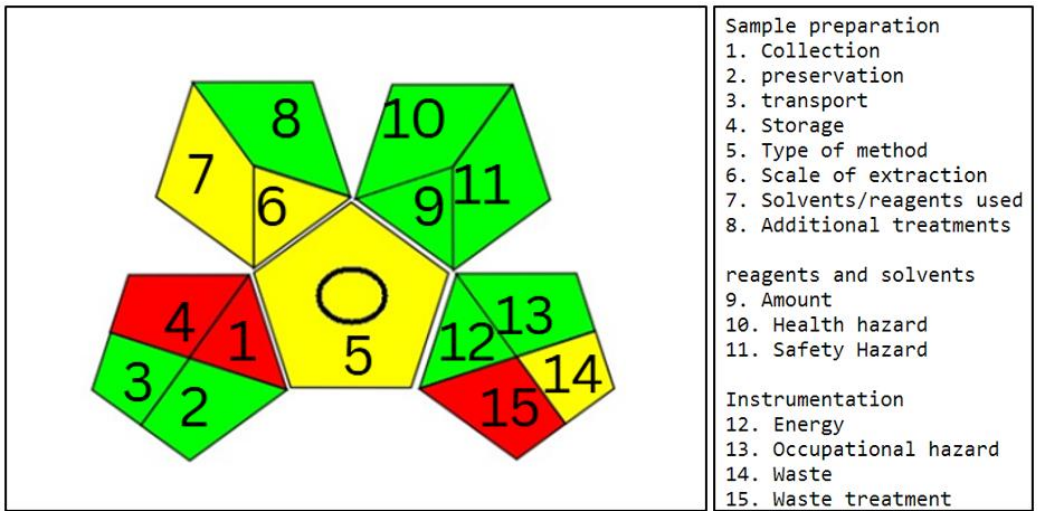



**Figure 12.** Greenness evaluation by GAPI.

The section's width conveys the weight of each code, and the procedure's efficiency is denoted by the transparent scale (red, yellow, and green). This easy-to-use program automatically creates a basic evaluation report. The greenness of the procedure should be near

1 if the value is higher. If the value is close to zero, the technique will be refused. It has greenness scores of 0.86. The outcome indicates that their color representation is green and their scores are closer to 1. The results show that the HPLC method's greenness values fall within the acceptable range (Figure 13).

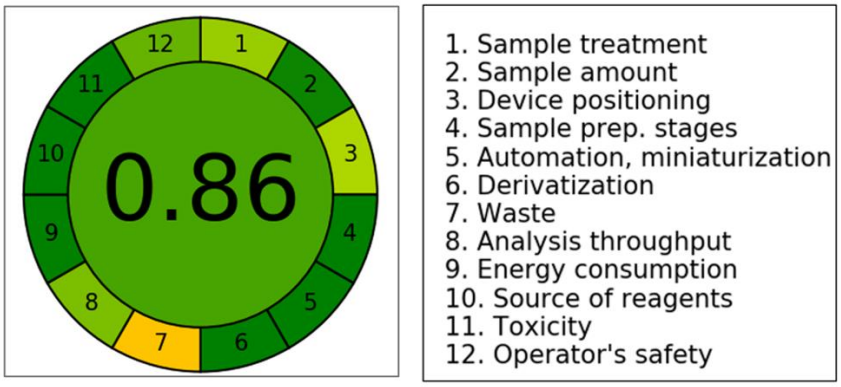



**Figure 13.** Greenness evaluation by AGREE.

The BAGI analytical tool played a crucial role in the development and validation of the HPLC method for the simultaneous estimation of Ivabradine Hydrochloride and Carvedilol. Providing a numerical assessment facilitated an objective evaluation of method performance, helping optimize critical parameters for improved precision, accuracy, and robustness. The numerical values obtained from the BAGI analysis (Figure 14) enabled a systematic comparison of different method conditions, thereby ensuring the selection of an optimal, well-validated approach. With greenness ratings close to 1.0 in BAGI, it showed excellent ecological compatibility and little environmental impact. This structured assessment strengthened the method's reliability and compliance with regulatory standards, making BAGI a valuable tool for analytical method development in pharmaceutical research.

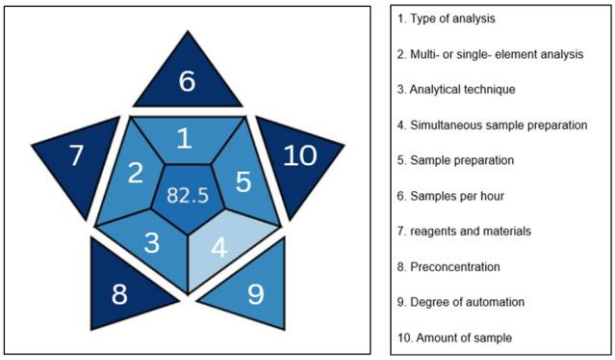



**Figure 14.** Greenness evaluation by BAGI.

*3.3. Discussion.*

The developed method showed consistent performance in system suitability parameters, including retention time, resolution, theoretical plates, and peak symmetry. Mechanistically, the low %RSD values observed can be attributed to the optimized interaction between analytes and the stationary phase, achieved through careful selection of mobile phase pH and composition. The robustness trials showed that minor variations in flow rate and pH did not significantly affect peak shape or resolution. This can be explained by the buffering capacity of acetate and the optimized polarity balance of the acetonitrile buffer system, which

ensured stable analyte partitioning and minimized band broadening. The results confirm the ruggedness and reproducibility of the method under real-world variations.

The AGREE score (0.86) and GAPI evaluation indicate that the developed method aligns strongly with Green Analytical Chemistry principles. Compared to traditional non-QbD HPLC methods reported by Dey *et al*. [12], Rao *et al*. [17], and Seerapu *et al*. [18], which use longer columns, higher buffer volumes, and longer run times, the proposed method demonstrates a lower environmental footprint. Lower solvent consumption and shorter analysis time not only improve efficiency but also minimize chemical waste. The high AGREE score reflects both the reduced hazard posed by reagents and the energy-saving features of the method, including ambient-temperature operation and short runtime.

## 4. Conclusions

This study successfully developed and validated a novel RP-HPLC method for the simultaneous estimation of Carvedilol and ivabradine hydrochloride using a Quality by Design approach. The application of Central Composite Design enabled the optimization of critical parameters such as run time, flow rate, and buffer composition, achieving robust and efficient separation within an 8-minute run time. The method demonstrated excellent resolution, minimal peak tailing, and high reproducibility. Validation results confirmed compliance with ICH guidelines, ensuring precision, accuracy, linearity, and robustness. The integration of QbD principles allowed a systematic risk assessment and ensured the method's adaptability for routine pharmaceutical quality control. Additionally, green analytical chemistry principles were incorporated to enhance sustainability. Evaluations using GAPI, AGREE, and BAGI tools highlighted the method's eco-friendly profile, with reduced solvent consumption and minimal environmental impact. This integration of QbD and green principles supports both analytical performance and environmental stewardship, making the method a valuable tool for sustainable pharmaceutical practices.

The developed RP-HPLC method for the simultaneous estimation of Carvedilol and Ivabradine Hydrochloride was found to be accurate, precise, robust, and environmentally friendly. The method showed excellent linearity and system suitability, with validated parameters meeting ICH guidelines.

Owing to its short run time, minimal solvent consumption, and reliable performance, the method is highly suitable for routine quality control analysis and can be applied in stability testing of pharmaceutical formulations.

In the future, this method can be scaled up or adapted for high-throughput environments and potentially transferred to other structurally similar beta blockers or cardiovascular analytes with minor modifications. This flexibility enhances its utility in both research and industrial settings.

## Author Contributions

Conceptualization – A.P., R.J.; Methodology – A.P.; Software – R.J.; Validation – A.P., R.J., A.J.; Formal analysis – A.P.; Investigation – A.P.; Resources – A.P.; Data curation – A.P.; Writing – original draft preparation – A.P.; Writing – review & editing – A.P.; Visualization – P.G., B.M.; Supervision – P.J.; Project administration – A.P., R.J., A.J. All authors have read and agreed to the published version of the manuscript.

## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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## Conflicts of Interest

The authors declare no conflict of interest.

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