ABSTRACT
A simple, sensitive, precise, specific, rapid, accurate, and novel reverse phase high performance liquid chromatography (RP-HPLC) method for determining carvedilol (CAR) and ivabradine (IVA) in bulk and its formulation has been developed validated. RP-HPLC performed the chromatographic separation on column C18 (4.6 mm x 2.5 cm, 5 µm) using acetonitrile:buffer pH 2.0 (60:40) pH of this buffer was adjusted to 2.0 with ortho-phosphoric acid, as a mobile phase. The flow rate was fixed at 0.90 mL/min. UV detection was operated at 275 nm, and injected volume was 20 µL. The retention time was found to be 2.931 for ivabradine and 3.370 for carvedilol. The RSD for ivabradine and carvedilol’s precision is within a limit of less than 2%, which indicates that the given method is highly precise.

Regarding the accuracy, the percentage recovery of the drug ivabradine is 99.48, and 98.19% for carvedilol, linearity of carvedilol and ivabradine ranged from 25–100 ppm and 20–80 ppm, respectively. The calibration curve shows good range and linearity. The correlation coefficient of carvedilol and ivabradine was 0.9987 and 0.9991, respectively. Limit of detection (LoD) and Limit of quantitation (LoQ) were found to be 3.79 ppm and 11.50 ppm for carvedilol and 2.47 ppm and 7.48 ppm for ivabradine, respectively. The acid, base, UV, and thermal stress studies presented the formation of a variety of degradation products; the given method showed good accuracy, linearity, precision, and robustness for analyzing the drug combination in bulk and its pharmaceutical formulations.

Keywords: Carvedilol, Ivabradine, Method development and validation, RP-HPLC, Stability study.

INTRODUCTION
Carvedilol is an antihypertensive drug chemically; it is named 1-(9H-Carbazol-4-yl-oxo)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol.1,3 It is a third-generation non-selective beta blocker that competitively blocks beta 1, beta 2, and alpha 1 adrenoreceptor.4,5 It is also used for the treatment of hypertension, CHF (congestive heart failure), and ischemic heart diseases (Figure 1).2,5

It is white or almost white solid powder at room temperature6, and which is completely soluble in DMSO (Dimethyl sulfoxide), methanol, sparingly soluble in isopropanol and ethanol, and slightly soluble in ethyl ether; practically insoluble in water and dilute acidic solution.3

Ivabradine is a cardiac medication chemically it is named as 3-[[3-[(7S)-3,4-dimethoxy-7-bicyclo [4.2.0] octa-1,3,5-trienyl] methyl-methylamino] propyl]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one (Figure 2).7,8 It reduces heart rate and use in treating heart failure patients. Ivabradine is selectively inhibited, if (funny channel), located in a sinoatrial node which controls the diastolic depolarization.9-11

It is white-slightly yellow powder it is soluble in some organic solvent such as ethanol, Dimethyl sulfoxide (DMSO), dimethyl formamide.
According to a literature survey, some analytical method has been developed and published to determine carvedilol and Ivabradine. So, there is a need for the development of a single method for the simultaneous determination of both drugs. This research paper describes the Reverse Phase High-Performance Liquid Chromatographic method procedure (RP-HPLC), which is accurate, reliable, sensitive, and stability, indicating the method for determination of Carvedilol and Ivabradine.

MATERIAL AND METHOD

Instruments, chemicals, APIs, and formulations have been mentioned with details in the tables 1 to 4.

(A) Selection of Mobile Phase

Various mobile phases were tried in different ratios for the selection of mobile phase. The drug carvedilol and ivabradine was varied mobile phases and were injected at different ratios and flow rates until a sharp peak with no interference peak containing spectrum was obtained. The varied mobile phases contained either one or two or three of the following solvents: acetonitrile, water and methanol. Tried at different ratios no favorable results were obtained. But the mobile phase containing acetonitrile and buffer pH 2.0 (phosphate buffer adjusted to 2.0 with ortho-phosphoric acid) were mixed in 60:40 ratio to give acceptable peak.

(B) Preparation of Mobile Phase

Mobile phase prepared by using acetonitrile and buffer pH 2.0 (Buffer pH 2.0–1.36 g of monobasic potassium phosphate was weighed. Mixed with 500 mL of HPLC grade water. pH of this solution was adjusted to 2.0 with ortho-phosphoric acid, mixed well) and then filtered and sonicated for 10 minutes.

(C) Diluent Preparation

Mobile phase use as a diluent.

(D) Standard Stock Solution

Precisely weighed 100 mg of carvedilol and ivabradine, transferred into two different 100 mL volumetric flasks containing mobile phase and the volume was made up to the mark with same which gives the stock solution having concentrations 1000 µg/mL.

(E) Preparation of Working Standard Solution

5.0 mL from Carvedilol stock solution and 4.0 mL from Ivabradine stock solution, transferred into 100 mL volumetric flask and made up the volume with mobile phase to acquire the concentration of working standard stock solution as 50 µg/mL of carvedilol and 40 µg/mL of ivabradine.

(F) Preparation of Sample Solution

20 tablets were weighed. avg. wt. = 0.1576 g, label claim-6.25 mg carvedilol and 5 mg ivabradine An accurately weighed amount of the finely crushed tablets equivalent to 125 mg of carvedilol and 100 mg of ivabradine was taken and transferred into a 100 mL volumetric flask; 60 mL of diluent was added and sonicated with occasional shaking for 10 minutes. The solution was diluted to volume with the mobile phase. The resultant solution was filtered through 0.2 µL syringe filter.

(G) Selection of Analytical Wavelength

A detection wavelength of 275 nm was selected for simultaneous estimation of both ivabradine and carvedilol based on common wavelength maxima from the individual compound spectral information.

(H) System Suitability Test

Details mentioned in Table 5.

(I) Optimize Chromatographic Condition

Details mentioned in Figure 3.
The developed RP-HPLC method was validated according ICH guidelines for the parameters like accuracy, linearity, precision, recovery, and robustness. The method validation included the following steps:

**Linearity**
The ability of a test process to produce test results proportional to the concentration (quantity) of analyte in the sample (within a specific range) is known as linearity. Linearity was studied by diluting the volume of standard stock solution (1000 μg/mL). Calibration curves were plotted by taking concentration on X axis and mean area on Y axis for carvedilol and ivabradine separately. Linear relationship was established between concentration and mean area and the same was confirmed by the regression coefficient obtained. Therefore, it was stated from those results of the current study that the linearity experiment was successful for carvedilol within a range of 25 to 100 μg/mL and for ivabradine within a range of 20 to 80 μg/mL.

**Limit of Detection (LoD) and Limit of Quantitation (LoQ)**
The determination of LoD and LoQ was done based on the calibration curve. Y-intercept i.e., slope of the standard deviation, was used to compute the LoD and LoQ of the given method.

**Precision**
Precision is the assessment of how close the records are to each other for a number of measurements under the same experimental conditions. The precision of the method was established for intra-day (repeatability), and inter-day precision was studied by diluting the volumes 40, 70 and 90 μg/mL for carvedilol and 30, 55, and 75 μg/mL for Ivabradine. Good result and %RSD value found within a limit.

**Robustness**
It is the capability of a method to stay unaffected by small but purposeful changes in method parameter. Experiments were performed for 50 μg/mL concentration of carvedilol and 40 μg/mL of ivabradine by changing conditions such as flow rate ratio (± 0.1 mL/min) and wavelength (+ 2 nm).

**Force Degradation Study**
As per ICH guidelines and common industry practice, forced degradation is usually performed in different stress conditions, i.e., acid, alkali, thermal, and UV, along with a control sample.

**Studies of Acid Degradation**
10 mg each of ivabradine and carvedilol were taken in separate beakers, and 10 mL of 1 N HCl was added into the individual beaker and kept on a water bath for hydrolysis for 4 hours at 80°C. After 4 hours of hydrolysis the samples were properly diluted and analyzed using HPLC. The separation of degraded products efficiently.

**Studies of Alkali (base) Degradation**
10 mg each of carvedilol and ivabradine were taken in separate beakers and 10 mL of 1 N NaOH was added into the individual beaker and kept on water bath for hydrolysis for 4 hours at 80°C. After 4 hours of hydrolysis the samples had been properly diluted and analyzed using HPLC. The separation of degraded products efficiently.

**Studies of UV Degradation**
For UV degradation 50 mg of carvedilol and ivabradine were exposed to ultraviolet radiation for 24 hours in UV chamber at 254 nm. No change in appearance was observed in both samples. After 24 hours, the samples were properly diluted and analyzed. The separation of degraded products efficiently.

**Studies of Thermal Degradation**
For thermal studies, the samples were kept in a drying oven at 150°C for 4 hours. After exposure, the samples were cooled, suitability diluted, and analyzed. No change in appearance was observed in both samples. The separation of degraded products efficiently.

**RESULT AND DISCUSSION**

**Assay**
Results attained were found within the acceptable limit of pharmacopeial standards for carvedilol and ivabradine (95–105% w/w) (Table 7).
RP-HPLC Method Development and Validation of Carvedilol and Ivabradine

**Linearity**

Carvedilol and ivabradine showed good coefficient calibration curves in the given concentration range of 25–100 ppm for carvedilol and 20–80 ppm for ivabradine, respectively (Figure 4 and 5, Table 8).

**LoD and LoQ**

LoD and LoQ were found to be 3.79 ppm and 11.50 ppm for carvedilol and 2.47 ppm and 7.48 ppm for ivabradine, respectively (Table 9).

**Accuracy**

From the result shown in the accuracy table, the recovery value of pure drugs was between 98 to 102%. Mean recovery was found for ivabradine is 99.48 and 98.19% for carvedilol. The result are shown in Tables 10 and 11.

**Precision**

The result of precision- the relative standard deviation value for inter-day or inter-day precision was less than 2%. The results show in Tables 12 and 13.

**Robustness**

The result of robustness were found to be satisfactory within range. %RSD of change in wavelength was found 1.37% for carvedilol and 1.34% for ivabradine.

%RSD of change in flow rate was found for carvedilol is 0.54% and 1.51% for ivabradine. The result is shown in Table 14 and 15.

**Force Degradation Studies**

As per ICH guidelines force degradation studies can be evaluated. The standard drug was degraded in to acid, base, thermal and photolytic etc. stability studies are used for developing the dosage form, selecting packaging, storage condition etc. the typical chromatogram of degradation study was show in Figure 6–13 and result of degradation shown in Table 16.

**Acid Degradation Study**

Degradant obtained for carvedilol is given in Figure 6 at different RT values for carvedilol at 2.579, 2.839, 3.387.

Degradant obtained for ivabradine is given in Figure 7 at different RT values for Ivabradine at 2.397, 2.584, 2.733, 2.949, 3.371, 4.014.
**Table 10**: Result of % recovery of ivabradine.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Standard stock solution</th>
<th>Sample stack solution</th>
<th>Diluted with mobile phase</th>
<th>Final concentration (ppm)</th>
<th>Amount recovered</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2</td>
<td>4</td>
<td>100</td>
<td>60</td>
<td>60.96</td>
<td>101.60</td>
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<td>100</td>
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<td>4</td>
<td>100</td>
<td>80</td>
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<td>98.87</td>
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<tr>
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<td>6</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>97.88</td>
<td>97.88</td>
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</tbody>
</table>

**Table 11**: Result of % recovery of carvedilol

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Standard stock solution</th>
<th>Sample stack solution</th>
<th>Diluted with mobile phase</th>
<th>Final concentration (ppm)</th>
<th>Amount recovered</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2</td>
<td>4</td>
<td>100</td>
<td>75</td>
<td>74.09</td>
<td>98.79</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>96.77</td>
<td>96.77</td>
</tr>
<tr>
<td>150</td>
<td>6</td>
<td>4</td>
<td>100</td>
<td>125</td>
<td>123.77</td>
<td>99.02</td>
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</tbody>
</table>

**Table 12**: Result of inter-day precision for carvedilol and ivabradine.

<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration (ppm)</th>
<th>Peak area</th>
<th>Mean</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td></td>
</tr>
<tr>
<td>Ivabradine</td>
<td>30</td>
<td>344045</td>
<td>344337</td>
<td>344559</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>651250</td>
<td>651365</td>
<td>651612</td>
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<tr>
<td></td>
<td>75</td>
<td>851958</td>
<td>851952</td>
<td>851765</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1242544</td>
<td>1243328</td>
<td>1230365</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>70</td>
<td>2206071</td>
<td>2206375</td>
<td>2205780</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2751716</td>
<td>2751807</td>
<td>2050487</td>
</tr>
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</table>

**Table 13**: Result of intraday precision for carvedilol and ivabradine.

<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration (ppm)</th>
<th>Peak area</th>
<th>Mean</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning</td>
<td>Afternoon</td>
<td>Evening</td>
<td></td>
</tr>
<tr>
<td>Ivabradine</td>
<td>30</td>
<td>344045</td>
<td>344378</td>
<td>343673</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>651250</td>
<td>651437</td>
<td>651605</td>
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<tr>
<td></td>
<td>75</td>
<td>851958</td>
<td>851520</td>
<td>851167</td>
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<tr>
<td></td>
<td>40</td>
<td>1242544</td>
<td>1242808</td>
<td>1243021</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>70</td>
<td>2206071</td>
<td>2206359</td>
<td>2206016</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2751716</td>
<td>2750591</td>
<td>2748855</td>
</tr>
</tbody>
</table>

**Table 14**: Result of change in wavelength for carvedilol and ivabradine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Wave length</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvedilol</td>
<td>273</td>
<td>1635702</td>
<td>1651737.5</td>
<td>22677.62</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>277</td>
<td>1667773</td>
<td>1667773</td>
<td>1667773</td>
<td>1.37</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>273</td>
<td>487547</td>
<td>492209</td>
<td>6593.06</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>277</td>
<td>496871</td>
<td>496871</td>
<td>496871</td>
<td>1.34</td>
</tr>
</tbody>
</table>

**Table 15**: Result of change in flow rate for carvedilol and ivabradine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Flow rate</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvedilol</td>
<td>0.8 mL/min</td>
<td>1647797</td>
<td>1654080</td>
<td>8885.50</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>1.0 mL/min</td>
<td>1660363</td>
<td>1660363</td>
<td>1660363</td>
<td>1.51</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>0.8 mL/min</td>
<td>508284</td>
<td>502924.5</td>
<td>7579.47</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>1.0 mL/min</td>
<td>497565</td>
<td>497565</td>
<td>497565</td>
<td>1.51</td>
</tr>
</tbody>
</table>
RP-HPLC Method Development and Validation of Carvedilol and Ivabradine

Table 16: Result of force degradation studies for carvedilol and ivabradine.

<table>
<thead>
<tr>
<th></th>
<th>Carvedilol</th>
<th>Ivabradine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress conditions</td>
<td>Assay%</td>
<td>Degradation%</td>
</tr>
<tr>
<td>Acid degradation</td>
<td>97.48</td>
<td>2.52</td>
</tr>
<tr>
<td>Base degradation</td>
<td>77.969</td>
<td>22.031</td>
</tr>
<tr>
<td>UV degradation</td>
<td>93.230</td>
<td>6.77</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>95.093</td>
<td>4.907</td>
</tr>
</tbody>
</table>

Figure 8: Chromatogram of base degradation- carvedilol

Figure 9: Chromatogram of base degradation- ivabradine

Figure 10: Chromatogram of thermal degradation- carvedilol

Figure 11: Chromatogram of thermal degradation- ivabradine

Base Degradation Study
Degrant obtained for carvedilol is given in Figure 8 at different RT values for carvedilol at 2.332, 3.154, 3.380, 3.621.
Degrant obtained for ivabradine is given in Figure 9 at different RT values for ivabradine at 2.383, 2.729, 2.956, and 3.376.

Thermal Degradation
Degrant obtained for carvedilol is given in Figure 10 at different RT values for carvedilol at 2.566, 3.364.
Degrant obtained for ivabradine is given in Figure 11 at different RT values for ivabradine at 2.528, 2.730, 2.944, 3.368.

UV Degradation
Degrant obtained for carvedilol is given in Figure 12 at different RT values for carvedilol at 2.944, 3.146, 3.366.
Degrant obtained for ivabradine is given in Figure 13 at different RT values for ivabradine at 2.533, 2.738, 2.945, 3.369.

CONCLUSION
The proposed method describes a rapid, precious, simple, accurate, and stable HPLC method for estimating carvedilol and ivabradine in bulk, and its formulation has been developed and validated. The method showed high theoretical plate and low telling factor. Based on the obtained result, it is concluded that the method is suitable for estimating these drugs. The linearity and correlation coefficient were found within range. The percentage recovery and precision were found within acceptable limits. Changes in wavelength and mobile phase variation do not affect the %RSD of the standard. LoD and LoQ were found within acceptable limits. The force degradation studies were performed with acid, base, UV,
and thermal conditions. The result of the method shows high stability. So developed method can be used to regularly analyze carvedilol and Ivabradine in bulk and their formulation.

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This research did not receive any specific grant from funding agencies in the commercial, public, or not-for-profit sector

**CONFLICT OF INTEREST**
Authors do not have any conflict of interest

**ETHICAL APPROVAL**
As no animal study is involved so, ethical approval is not required.

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**AUTHORS CONTRIBUTION**
PBC conceived and designed the study, conducted research, and provided researched materials. Analyzed and interpreted data. KVD collected and organized data. MHK wrote the article's initial and final draft, provided logistic support, and reviewed the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

**REFERENCE**