RESEARCH ARTICLE

Stability Indicating Method Development and Validation of Carvedilol and Ivabradine in Bulk and its Formulation by Reverse Phase High Performance Liquid Chromatography Method

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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.

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INTRODUCTION

Carvedilol is an antihypertensive drug chemically; it is named 1-(9H-Carbazol-4-yloxy)-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 temperature[6], 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.

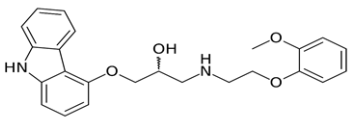

Figure 1: Structure of Carvedilol

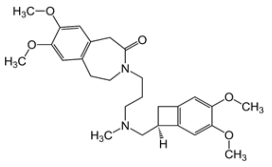

Figure 2: Structure of Ivabradine

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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: acetonitrileacetonitrile, water and methanol. Tried at different ratios no favorable results were obtained. But the mobile phase containing acetonitrileacetonitrile 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.

Table 1: List of instrument use

S. no	*Name*	*Model*	*Manufacturer/supplier*
1	Analytical weighing balance	14TR 220E	ESSAE, VIBRA+
2	Sonicator		
3	pH meter	PN16380313	LABINDIA, PHAN
4	1 MLH stirrer with Hot plate		REMI
5	High-performance liquid chromatography	LC 20 AD	SHIMADZU, JAPAN.
6	Water bath		OSWORLD
7	Drying oven		OSWORLD

Table 2: List of chemical use

S. no.	*Name of chemical*	*Specification*	*Manufacturer/ supplier*
1	Acetonitrile	HPLC Gradient	Rankem
2	Water	HPLC	Molychem
3	Ortho phosphoric acid	SQ	Qualigens
4	Potassium dihydrogen orthophosphate	AR	Merck
5	Methanol	HPLC	Rankem

Table 3: List of API use

S. no.	*Name of drug*	*Specification*	*Manufacturer/supplier*
1	Carvedilol	Working standard	Swapnroop Drugs Pvt. Ltd. Aurangabad
2	Ivabradine	Working standard	Swapnroop Drugs Pvt. Ltd. Aurangabad

(B) Preparation of Mobile Phase

Mobile phase prepared by using acetonitrileacetonitrile 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.

Table 4: List of formulation use

S. no	*Name of chemical*	*specification*	*Manufacturer/ supplier*
1	Cardivas IN 6.25/5	Working standard	Sun Pharma Ltd.

Table 5: System suitability parameter

System suitability parameter	*Ivabradine*	*Carvedilol*
Retention Time	2.931	3.370
Theoretical plates	3372	4186
Asymmetry	1.241	1.253
Resolution	000	2.140

Table 6: Optimize chromatographic condition

Mobile phase	*Acetonitrile: Buffer pH 2.0 (60: 40)*
Column	C18, 4.6 mm x 2.5 cm, 5 µm (Make-Shimadzu)
Flow rate	0.90 mL/min
Injection volume	20 µL
Wavelength	275 nm
Run time	8 minutes
HPLC Make	Shimadzu

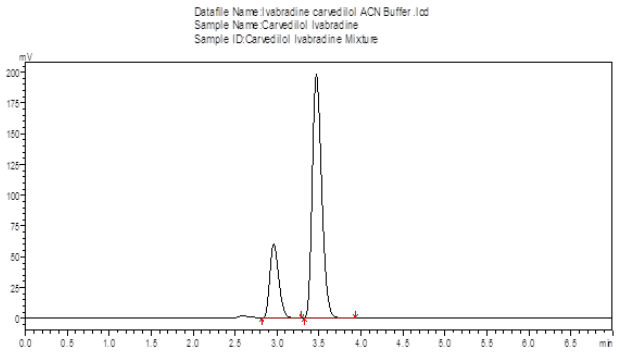

Figure 3: Chromatogram of ivabradine and carvedilol.

(J) Method Validation

The developed RP-HPLC method was validated according ICH guidelines for the parameters like accuracy, linearity, precision, recovery, and robustness.[12-15,22]

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.

Accuracy

It is defined as the closeness between the true value and the observed value. Sometimes it is also called trueness. Accuracy was calculated from the results of a precision experiment, and the results obtained for percent accuracy at three levels across the range are tabulated. According to ICH guideline Q2R1, accuracy is determined across the range at three concentration levels (QC standards).

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).

(K) 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.[16-21]

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 80ºC. 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 80ºC. After 4 hours of hydrolysis the samples had been properly diluted and analysed 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 150ºC 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).[22]

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.

Table 7: Assay data for carvedilol and ivabradine

Drug name	*Composition (ppm)*	*Area of standers*	*Area of sample*	*% Essay*
Ivabradine	40	451446	456519	101.12%
Carvedilol	50	1553920	1577362	101.50%

Table 8: Result of linearity

Drug	*Conc. (ppm)*	*Area*
Carvedilol	25	791460
	30	936727
	50	1553283
	60	1886020
	75	2238938
	100	3009324
	Correlation coefficient	0.9987
	20	228978
Ivabradine	25	289592
	40	451330
	50	567132
	60	700352
	80	937642
	Correlation coefficient	0.9991

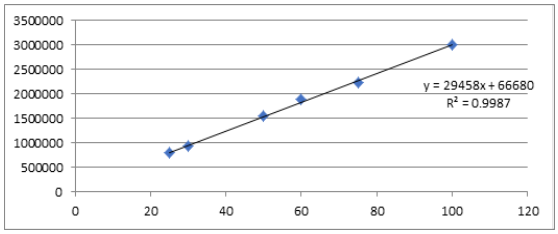

Figure 4: Linearity curve for carvedilol.

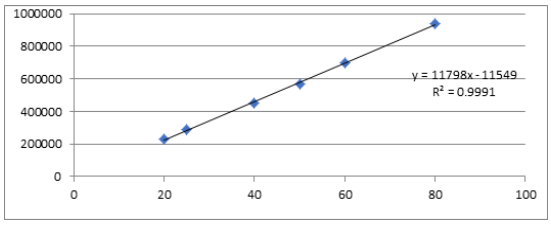

Figure 5: Linearity curve for ivabradine.

Table 9: Summary of validation data.

Analytical parameter	*Carvedilol*	*Ivabradine*
Linearity range (ppm)	25-100	20-80
Regression equation	y = 29458x + 66680	y = 11798x - 11549
Correlation coefficient (r^2)	R^2 = 0.9987	R^2 = 0.9991
Limit of detection	3.79	2.47
Limit of quantitation	11.50	7.48

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.

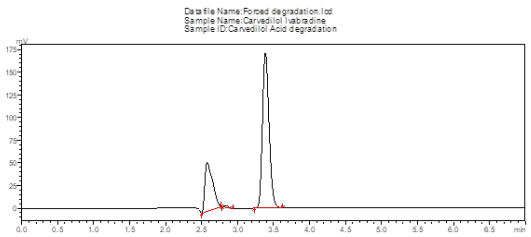

Figure 6: Chromatogram of acid degradation- carvedilol.

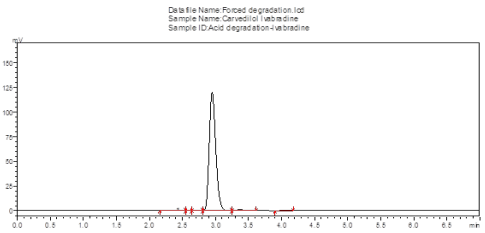

Figure 7: Chromatogram of acid degradation- ivabradine.

Table 10: Result of % recovery of ivabradine.

Concentration (%)	*Standard stock solution*	*Sample stack solution*	*Diluted with the mobile phase*	*Final concentration (ppm)*	*Amount recovered*	*%Recovery*
50	2	4	100	60	60.96	101.60
100	4	4	100	80	79.18	98.87
150	6	4	100	100	97.88	97.88

Table 11: Result of % recovery of carvedilol

Concentration (%)	*Standard stock solution*	*Sample stack solution*	*Diluted with mobile phase*	*Final concentration (ppm)*	*Amount recovered*	*%Recovery*
50	2	4	100	75	74.09	98.79
100	4	4	100	100	96.77	96.77
150	6	4	100	125	123.77	99.02

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

Name	*Concentration (ppm)*	*Peak area*			*Mean*	*%RSD*
		Day 1	Day 2	Day 3		
	30	344045	344337	344559	344280.3	0.06
Ivabradine	55	651250	651365	651412	651342	0.01
	75	851958	851952	851765	851891.6	0.01
	40	1242544	1243328	1230365	1241745	0.17
Carvedilol	70	2206071	2206375	2205780	2206075	0.01
	90	2751716	2751807	2050487	2751336	0.03

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

Name	*Concentration*	*Peak area*			*Mean*	*%RSD*
		Morning	Afternoon	Evening		
	30	344045	344378	343673	344032	0.10
Ivabradine	55	651250	651437	651605	651430	0.03
	75	851958	851520	851167	851548	0.05
	40	1242544	1242808	1243021	1242791	0.02
Carvedilol	70	2206071	2206359	2206016	2206382	0.01
	90	2751716	2750591	2748855	2750387	0.05

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

Drug	*Wave length*	*Peak area*	*Mean*	*SD*	*%RSD*
Carvedilol	273	1635702	1651737.5	22677.62	1.37
	277	1667773			
Ivabradine	273	487547	492209	6593.06	1.34
	277	496871			

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

Drug	*Flow rate*	*Peak area*	*Mean*	*SD*	*%RSD*
Carvedilol	0.8 mL/min	1647797	1654080	8885.50	0.54
	1.0 mL/min	1660363			
Ivabradine	0.8 mL/min	508284	502924.5	7579.47	1.51
	1.0 mL/min	497565			

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

Ivabradine			*Carvedilol*		
Stress conditions	*Assay%*	*Degradation%*	*Stress conditions*	*Assay%*	*Degradation%*
Acid degradation	97.48	2.52	Acid degradation	72.514	27.486
Base degradation	77.969	22.031	Base degradation	47.653	52.347
UV degradation	93.230	6.77	UV degradation	99.73	0.27
Thermal degradation	95.093	4.907	Thermal degradation	98.995	1.005

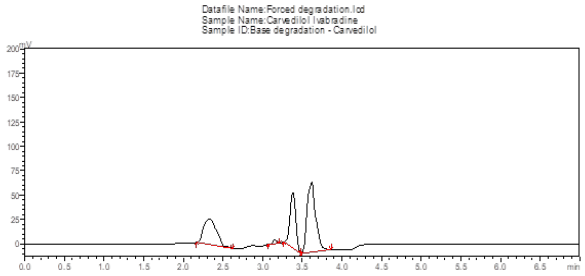

Figure 8: Chromatogram of base degradation- carvedilol

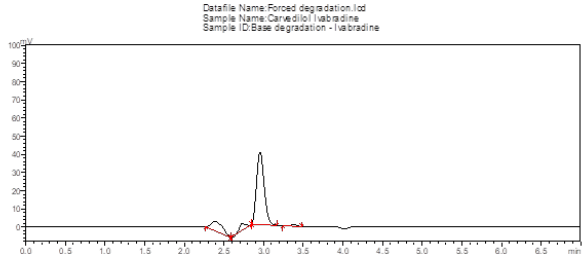

Figure 9: Chromatogram of base degradation- ivabradine

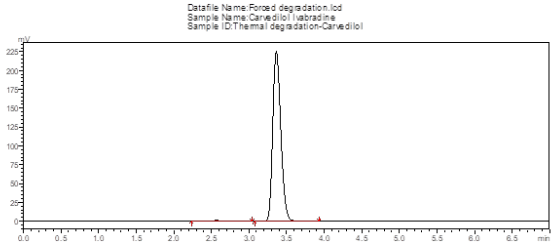

Figure 10: Chromatogram of thermal degradation- carvedilol

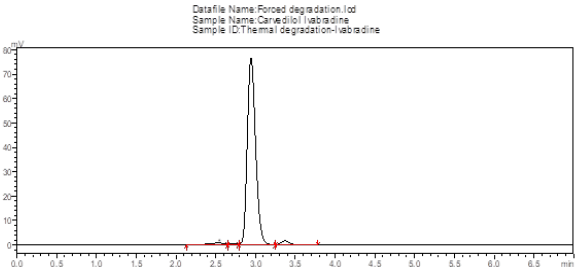

Figure 11: Chromatogram of thermal degradation- ivabradine

Base Degradation Study

Degradant obtained for carvedilol is given in Figure 8 at different RT values for carvedilol at 2.332, 3.154, 3.380, 3.621.

Degradant obtained for ivabradine is given in Figure 9 at different RT values for ivabradine at 2.383, 2.729, 2.956, and 3.376.

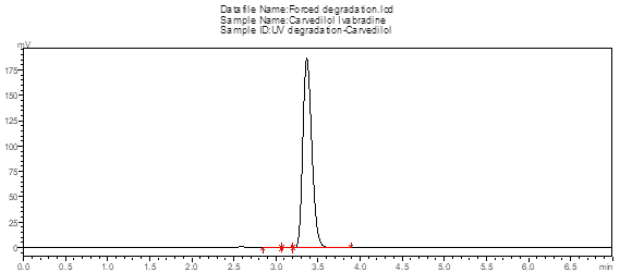

Figure 12: Chromatogram of uv degradation- carvedilol

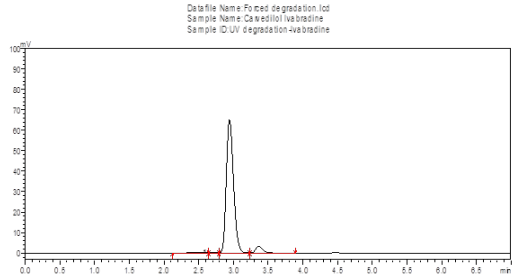

Figure 13: Chromatogram of uv degradation- ivabradine

Thermal Degradation

Degradant obtained for carvedilol is given in Figure 10 at different RT values for carvedilol at 2.566, 3.364.

Degradant 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

Degradant obtained for carvedilol is given in Figure 12 at different RT values for carvedilol at 2.944, 3.146, 3.366.

Degradant 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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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.

ACKNOWLEDGMENT

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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.

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