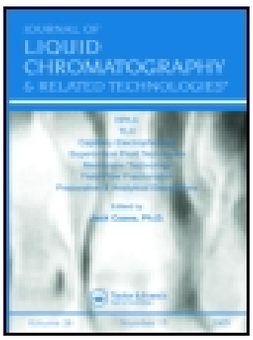

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Development and validation of UPLC method for simultaneous quantification of carvedilol and ivabradine in the presence of degradation products using DoE concept

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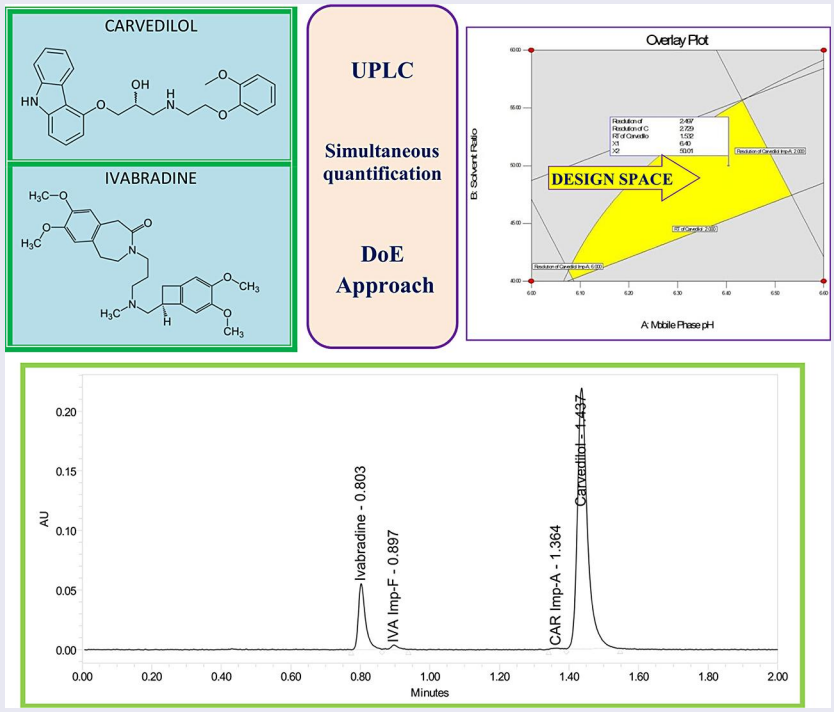
ABSTRACT

A methodical design-of-experiments were performed by applying quality-by-design concepts to establish a design-space for simultaneous and rapid quantification of Carvedilol and Ivabradine by UPLC in the presence of degradation products. Response-surface, central-composite design, and quadratic model were employed for statistical assessment of experimental data using the Design-Expert software. Response variables such as resolution and retention time were analyzed statistically for chromatographic screening. During DoE study, various plots such as perturbation, contour, 3D and design-space plots were considered for method optimization. The method was developed using C8 [100 × 2.1 mm, 1.8 µ] UPLC column, mobile phase comprising 0.5% triethylamine buffer [pH 6.4] and acetonitrile in the ratio of 50:50 v/v, the flow rate of 0.4 mL minute^{-1} and UV detection at 285 nm for both Carvedilol and Ivabradine. The method was developed with a short run time of two minutes. The method was found to be linear in the range of 25.0–199.9 µg mL^{-1} and 8.9–21.3 µg mL^{-1} for Carvedilol and Ivabradine, respectively with a correlation coefficient of 0.9998 in each case. The recovery values were found in the range of 99.7–100.8% and 98.9–100.9% for Carvedilol and Ivabradine, respectively. The method was validated according to ICH Q2 (R1) guidelines.

GRAPHICAL ABSTRACT



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Introduction

Carvedilol is a nonselective beta-adrenergic blocking agent with alpha-1-blocking activity. It is chemically (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino] -2-propanol (Figure 1(a)). Carvedilol is a white to off-white powder with a molecular weight of 406.5 g mole^{-1} and a molecular formula of $C_{24}H_{26}N_2O_4$. Carvedilol is freely soluble in dimethylsulfoxide, soluble in methylene chloride and methanol.[1] Ivabradine oxalate is chemically (S)-7,8-dimethoxy-3-{3-{N-[(4,5-dimethoxybenzocyclobut-1-yl) methyl]-N-(methyl) amino}propyl}-1,3,4,5-tetrahydro-2H-3-benzazepin-2-one oxalic acid (Figure 1(b)). Ivabradine oxalate is a white to off-white, hygroscopic powder with a molecular weight of 558.6 g mole^{-1} and a molecular formula $C_{27}H_{36}N_2O_5$ $(COOH)_2$. Ivabradine oxalate is soluble in chloroform and sparingly soluble in methylene chloride.[2–3] The combination of Carvedilol and Ivabradine is used in the treatment of chronic stable angina pectoris in coronary artery disease and for treatment of chronic heart failure.[4] Carvedilol is having an impurity-A, which is chemically 1-[[9-[2-hydroxy-3-[[2-(2-methoxy phenoxy) ethyl] amino] propyl]-9H-carbazol-4-yl] oxy]-3-[[2-(2-methoxy phenoxy)ethyl] amino] propan-2-ol (Figure 1(c)). Ivabradine oxalate is having an Ivabradine impurity-F, which is chemically (S) -7, 8-dimethoxy-3-{3-{N-[(4,5-dimethoxy benzocyclobut-1-yl)-methyl]-N-(methylamino propyl}-4, 5-dihydro-2H-3-benzazepin-1,2-dione (Figure 1(d)). Carvedilol impurity-A is a process related impurity in Carvedilol. Ivabradine impurity-F is a major degradation impurity in the Ivabradine oxalate and this impurity mainly appears during stability.[5,6]

Carvedilol is official in the United States Pharmacopeia (USP) and European Pharmacopeia (Ph. Eur.), whereas Ivabradine is not official in any pharmacopeia. Literature review reveals that several analytical methods are reported for determination of Carvedilol and Ivabradine, either individually or combined with other drugs, in pharmaceutical dosage forms or in biological fluids by using analytical techniques such as spectrophotometry,[7] capillary electrophoresis,[8] HPLC,[9–18] HPTLC [19] and LCMS.[20,21] As per available literature, there is no validated ultra performance liquid chromatography (UPLC) method available using a design-of-experiments (DoE) concept, for simultaneous estimation of Carvedilol and Ivabradine in bulk drug and in pharmaceutical dosage forms.

A stability-indicating method is a quantitative analytical procedure used to detect a decrease in the amount of drug substance present due to degradation. According to FDA guidelines,[22,23] stability indicating method is defined as a validated analytical procedure that accurately and precisely measures active ingredients free from potential interferences like degradation products, process impurities, and excipients.

Design of experiments (DoE) study is a series of experiments, in which purposeful changes are made to input factors to identify causes for significant changes in the output responses. Design-Expert software is used to conduct the DoE studies for chromatography method optimization to develop a robust analytical method. The outcome of DoE studies is to obtain the design space graphically as a result of the multivariate statistical analysis, where within the design space allowable ranges of the method conditions can be derived based on predefined target specifications of the method.[24,25]

The key objective of the proposed research work is to develop a stability-indicating UPLC method using DoE concepts for simultaneous and rapid estimation of Carvedilol and Ivabradine in the presence of degradation products for bulk drugs, pharmaceutical tablet dosage forms and to validate the method as per ICH guideline ICH Q2 (R1).[26] Development of a method with shorter chromatographic run time (reduces the analysis time), low solvent utilization, cost-effective altogether increases the pharmaceutical productivity in routine quality control of the pharma industry.

Experimental

Materials and reagents

Carvedilol and Ivabradine oxalate working standards and film-coated tablets of Carvedilol and Ivabradine were provided

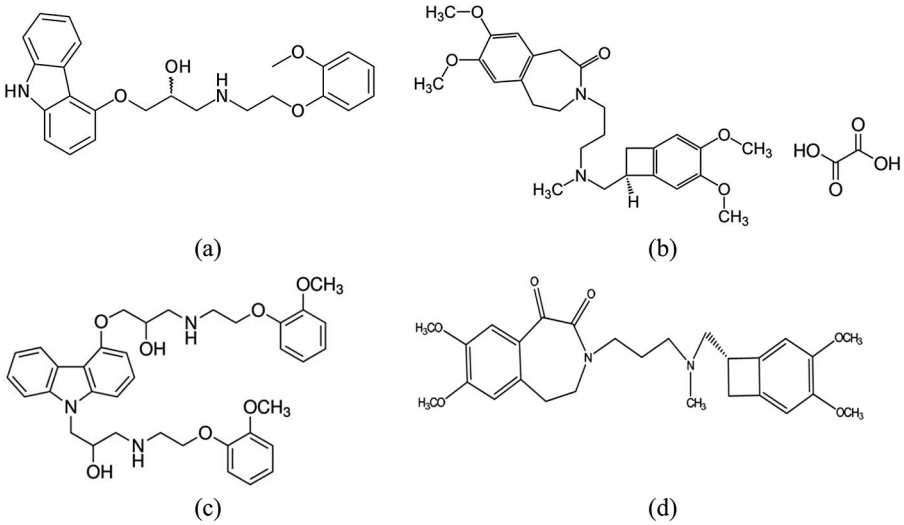

Figure 1. Molecular structure of (a) Carvedilol (b) Ivabradine oxalate (c) Carvedilol impurity-A and (d) Ivabradine impurity-F.

by AET Laboratories Pvt. Ltd., Hyderabad, India. Triethylamine, hydrochloric acid, sodium hydroxide, phosphoric acid, hydrogen peroxide, potassium dihydrogen phosphate, and potassium chloride of Emparta grade were purchased from Merck, India. Acetonitrile and methanol of HPLC grade were procured from Merck, India. Milli-Q-water was collected from Merck Millipore ELIX-10 system.

Instrumentation

UPLC system with Empower-3 software, Kromasil C8 100 × 2.1 mm, 1.8 µ and Acquity BEH C8 100 × 2.1 mm, 1.8 µ columns were used for chromatographic method optimization. Design-Expert version 8.0 (Stat-Ease) was used for DoE experimentation. Analytical balance (XP-205 DR model, Metler Toledo), rotary shaker (RS 24BL, REMI), pH meter (Orion Star A211, Thermo), water bath (MSI 8, Meta Lab), vacuum oven (Thermolab), vacuum filtration unit (Millivac-Maxi 230 V, Millipore), photostability chamber (NEC103RSPSI, Newtronics) and sonicator (9L250H, PCI) were used.

Chromatographic conditions

UPLC chromatographic conditions were optimized based on design-of-experiments (DoE) studies using the Design-Expert software. The chromatographic separation was achieved on C8 100 × 2.1 mm, 1.8 µ column using mobile phase composed of 0.5% v/v triethylamine buffer (pH 6.4) and acetonitrile in the ratio of 50:50 v/v. The mobile phase flow rate was set at 0.4 mL minute^{-1} and UV detection wavelength was set at 285 nm for both Carvedilol and Ivabradine. Injection volume was set at 1 µL with a column temperature of 30°C. Total chromatographic run time of the method was two minutes. A mixture of water and acetonitrile in the ratio of 50:50 v/v was used as a diluent.

Standard and sample preparation

Weighed and transferred about 62.5 mg of Carvedilol working standard, 22.5 mg of Ivabradine oxalate working standard into a 100 mL clean and dry volumetric flask. Added 60 mL of diluent, sonication was done to dissolve and made up to volume with diluent. From this solution, 5 mL was diluted to 25 mL with diluent to obtain a standard solution with a concentration of 125 µg mL^{-1} of Carvedilol and 45 µg mL^{-1} of Ivabradine oxalate. Taken 20 tablets of the test sample into a mortar and pestle, and then crushed to a fine powder. Weighed and transferred powder equivalent to 25 mg of Carvedilol and 7.5 mg of Ivabradine into a 200 mL clean and dry volumetric flask. Then added 150 mL of diluent and sonication was carried out for 10 minutes with intermittent shaking for extraction of the drug. The volume was made up to 200 mL with diluent. The sample solution was filtered through 0.2 µ PVDF syringe filter. Carvedilol and Ivabradine were quantified using following formulae where, 'A_x' was the area obtained from sample chromatogram, 'A_s' was the average area obtained from standard chromatograms, 'WC_{std}' was the weight of Carvedilol standard in mg, 'WI_{std}' was the weight of Ivabradine oxalate standard in mg, 'W_{spl}' was weight of test sample in mg, 'AW' was the average weight of drug product, 'LC' was the label claim of respective drug substance, and, 'PC' and 'PI' were the percentage assay of Carvedilol and Ivabradine oxalate standards, respectively on as is basis. 468.59 and 558.6 were the molecular weights of Ivabradine and Ivabradine oxalate respectively.

$$Carvedilol\ assay(\%) = \frac{A_x}{A_s} \times \frac{WC_{std}}{100} \times \frac{5}{25} \times \frac{200}{W_{spl}} \times \frac{AW}{LC(Carvedilol)} \times PC$$

$$Ivabradine\ assay(\%) = \frac{A_x}{A_s} \times \frac{WI_{std}}{100} \times \frac{5}{25} \times \frac{200}{W_{spl}} \times \frac{468.59}{558.6} \times \frac{AW}{LC(Ivabradine)} \times PI$$

Method validation

The developed UPLC method for simultaneous estimation of Carvedilol and Ivabradine was validated according to an international council for harmonization guideline ICH Q2 (R1) validation of analytical procedures.[26] The present method was validated for system suitability, specificity, linearity, accuracy, precision, intermediate precision and robustness parameters. The system suitability test was performed to verify whether the analytical system (UPLC system, analytical solutions, column, etc.) was suitable or not for producing accurate and consistent results. System suitability test was performed before analyzing the test samples. The system suitability of the proposed method was evaluated by calculating parameters such as the number of theoretical plates not less than 2000, tailing factor not more than 2.0 and percentage RSD from five standard injections not be more than 2.0.[27–31] The specificity of the analytical method was studied by verifying the interference of diluent, placebo, degradation impurity peaks at the retention time of Carvedilol and Ivabradine. Standard and sample solutions were prepared at a concentration of 125 µg mL^{-1} of Carvedilol and 45 µg mL^{-1} of Ivabradine oxalate. Placebo solution was prepared similarly as sample preparation by taking a placebo without drug substance. Solutions were injected into the chromatographic system by giving 200 to 400 nm wavelength range in PDA system. Recorded the chromatograms, verified for peak purity of Carvedilol and Ivabradine as well as interferences of the blank, placebo and degradation impurity peaks.

Forced degradation studies

Executed the forced degradation studies (FDS) and all the FDS samples were diluted with diluent after completion of the degradation process (Table 1). Blank and placebo solutions were prepared in a similar way in particular degradation in order to exclude any contribution from the process. Forced degradation sample solutions were injected into UPLC system and recorded the chromatograms. Peak purity was determined

Table 1. Specificity, forced degradation study results.

Sample name	Component	Retention Time (min)	Purity Angle	Purity Threshold	Peak Purity
Standard	Carvedilol	1.029	1.309	1.449	Pass
	Ivabradine	0.711	27.603	53.756	Pass
Sample-initial	Carvedilol	1.030	1.235	1.287	Pass
	Ivabradine	0.712	27.083	53.337	Pass
Acid degradation (0.1 N HCl, 60°C, 24 hrs)	Carvedilol	1.031	1.280	1.460	Pass
	Ivabradine	0.713	26.905	55.337	Pass
Alkali degradation (0.1 N NaOH, 60°C, 24 hrs)	Carvedilol	1.032	1.269	7.997	Pass
	Ivabradine	0.714	6.294	90.000	Pass
Oxidation (0.1% H2O2, 24 hrs)	Carvedilol	1.033	1.393	1.625	Pass
	Ivabradine	0.714	24.805	49.067	Pass
Photo degradation (1.2 million lux hrs, 200 watt hrs per square meter)	Carvedilol	1.032	1.372	1.494	Pass
	Ivabradine	0.714	26.072	49.666	Pass
Thermal degradation (70°C, 24 hrs)	Carvedilol	1.032	1.335	1.411	Pass
	Ivabradine	0.714	27.236	55.004	Pass
Water degradation (Water, 60°C, 24 hrs)	Carvedilol	1.032	1.252	1.402	Pass
	Ivabradine	0.714	28.916	57.154	Pass

for Carvedilol and Ivabradine in order to verify any interference of blank, placebo and degradation impurity peaks at the retention time of Carvedilol and Ivabradine.

Linearity and range

The linearity of the analytical method was its ability to obtain test results which were directly proportional to the concentration of an analyte in the test sample within a given range. The linearity of the analytical method was determined by preparing six concentration levels in the range of 25.0–199.9 µg mL^{-1} of Carvedilol and 8.9–71.0 µg mL^{-1} of Ivabradine oxalate. The correlation coefficient (R), regression coefficient (R^2), y-intercept and slope of regression line were

Table 2. System suitability evaluation, linearity and robustness results.

Parameter	Acceptance criteria[27–29]	Results of the test: Specificity	Results of the test: Precision	Remarks
Carvedilol				
% RSD of area/5 injections	Not less than 2.0	0.64	0.27	Satisfactory
USP tailing factor	Not more than 2.0	1.48	1.47	Satisfactory
Theoretical plates	Not less than 2000	10812	13694	Satisfactory
Ivabradine				
% RSD of area/5 injections	Not less than 2.0	0.30	0.47	Satisfactory
USP tailing factor	Not more than 2.0	1.55	1.58	Satisfactory
Theoretical plates	Not less than 2000	6947	8310	Satisfactory

Linearity test results

Type of test	Assay-Carvedilol	Assay-Ivabradine
Test concentration (µg mL^{-1})	25.0–199.9	8.9–71.0
Correlation coefficient [R]	0.9998	0.9998
Regression coefficient [R^2]	0.9997	0.9997
Slope	4444.2039	2188.6568
Intercept	3029.896	565.1676

Robustness results

Parameter	Change done	Carvedilol (%)	Ivabradine (%)	Remarks
Solution stability	Initial	101.5	100.3	Solutions are stable for 24 hours
	After 24 hours	99.9	101.7	
Wavelength (285 ± 2 nm)	285 nm	101.5	100.3	No significant variation in results
	287 nm	100.1	100.5	
	283 nm	101.2	101.6	
Flow rate (0.4 ± 0.1 mL min^{-1})	0.4 mL min^{-1}	100.0	98.6	
	0.5 mL min^{-1}	100.1	99.1	
	0.3 mL min^{-1}	100.7	99.8	
Column oven temperature (35 ± 5°C)	35°C	100.0	98.6	
	30°C	100.8	99.9	
	40°C	99.6	99.1	
Mobile phase buffer pH (6.4 ± 0.2)	pH 6.4	100.0	98.6	
	pH 6.2	99.8	99.9	
	pH 6.6	99.1	98.4	
Mobile phase ratio (Buffer: Acetonitrile, v/v)	50:50 v/v	100.0	98.6	
	45:55 v/v	99.2	98.5	
	55:45 v/v	98.9	97.7	

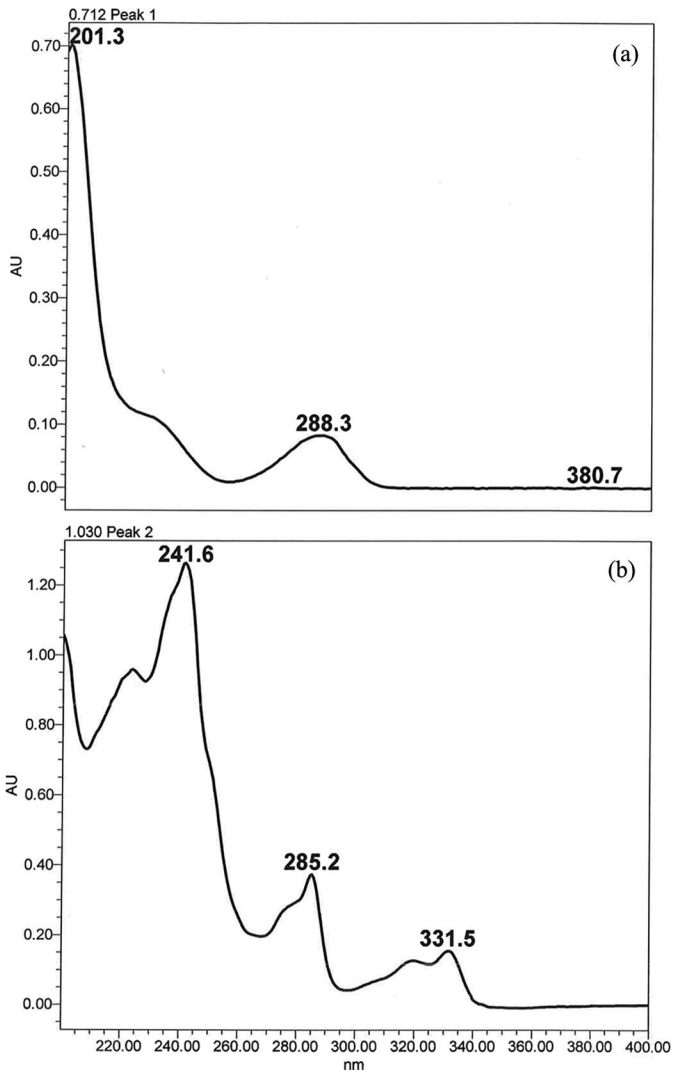

Figure 2. UV spectrums of (a) Ivabradine (b) Carvedilol.

calculated (Table 2). The range of the analytical method was proved by performing the precision, linearity, and accuracy at minimum 80% and maximum 160% concentration levels with respect to sample concentration. Weighed and transferred about 500 mg of Carvedilol and 178 mg of Ivabradine oxalate into a 100 mL volumetric flask. These substances were dissolved and diluted to volume with diluent to obtain a concentration of 5000 µg mL^{-1} of Carvedilol and 1780 µg mL^{-1} of Ivabradine oxalate (linearity stock). To prepare 20%, 40%, 80%, 100%, 120% and 160% levels, respectively, 1 mL, 2 mL, 4 mL, 5 mL, 6 mL and 8 mL of linearity stock solutions were diluted to 200 mL with diluent.

Accuracy

A known amount of Carvedilol and Ivabradine oxalate drug substances were spiked at 80%, 100%, 120%, and 160% level with respect to sample concentration to the placebo and analyzed by the proposed UPLC method. Percentage recoveries of Carvedilol and Ivabradine were determined. Weighed and transferred about 500 mg of Carvedilol and 178 mg of Ivabradine oxalate into a 100 mL clean and dry volumetric flask. Dissolved and diluted to volume with diluent to obtain a concentration of 5000 µg mL^{-1} of Carvedilol and 1780 µg mL^{-1} of Ivabradine oxalate (accuracy stock). Weighed

Table 3. Results of pH scouting studies and design of experiments.

Results of pH scouting studies						
Buffer pH	Name of the component	Retention time (minute)	Tailing factor	Plate count	Resolution between Ivabradine and Carvedilol	Resolution of Ivabradine and impurity-F
pH 2.0	Ivabradine	0.718	1.67	6753	7.37	Peak merged
	Carvedilol	1.023	1.65	8452		
pH 3.0	Ivabradine	0.722	1.66	6259	7.79	Peak merged
	Carvedilol	1.054	1.67	8697		
pH 4.0	Ivabradine	0.700	1.70	6452	8.83	Peak merged
	Carvedilol	1.064	1.72	9364		
pH 5.0	Ivabradine	0.759	1.59	7016	12.01	Peak merged
	Carvedilol	1.275	1.61	12406		
pH 6.0	Ivabradine	0.991	1.45	10001	17.08	3.73
	Carvedilol	1.874	1.41	15777		
pH 7.0	Ivabradine	0.810	1.53	7482	13.60	1.87
	Carvedilol	1.434	1.55	12867		

Design of experiments (DoE)		
Response variables	Control variables	Proposed ranges of design
Resolution of Ivabradine and impurity-F	Mobile phase composition (Buffer: acetonitrile)	Actual – 50:50 v/v High – 40:60 v/v Low – 60:40 v/v
Resolution of Carvedilol and impurity-A	Mobile phase pH	Actual – pH 6.3 High – pH 6.6 Low – pH 6.0
Retention time of Carvedilol	Column temperature	Actual – 35°C High – 40°C Low – 30°C

Table 4. Experimental results of DoE study and effect on response variables.

DoE study				Results of response variables		
Exp. no.	pH	Solvent (%)	Column temperature (°C)	Resolution between Ivabradine and impurity-F	Resolution between Carvedilol and impurity-A	Retention time of Carvedilol (minutes)
1	6.3	50	35	2.36	1.52	1.318
2	6.0	60	40	1.55	1.48	0.840
3	6.3	50	35	2.36	1.52	1.318
4	6.3	50	35	2.36	1.52	1.318
5	6.0	40	40	2.34	2.93	1.975
6	6.0	60	30	1.18	2.17	0.853
7	6.3	50	35	2.36	1.52	1.318
8	6.6	40	40	5.18	7.57	2.942
9	6.6	40	30	4.54	2.57	2.793
10	6.6	60	30	1.95	1.00	0.992
11	6.3	50	35	2.36	1.52	1.318
12	6.0	40	30	1.83	6.65	1.948
13	6.3	50	35	2.36	1.52	1.318
14	6.6	60	40	1.96	1.10	1.018

Observations from DoE study plots			
	Effect on response variables		
Increase of control variable	Resolution between Ivabradine and impurity-F	Resolution between Carvedilol and impurity-A	Retention time of Carvedilol
A: Mobile phase pH	Increases	Decreases	Increases
B: Solvent ratio	Decreases	Decreases	Decreases
C: Column temperature	No effect	Decreases	No effect

and transferred placebo equivalent to one tablet weight into a 200 mL clean and dry volumetric flask. Added 4 mL, 5 mL, 6 mL, and 8 mL of accuracy stock solution to obtain 80%, 100%, 120%, and 160% concentration levels respectively. Solutions were sonicated for 10 minutes with intermittent shaking and made up to volume with diluent.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Repeatability for assay method was demonstrated by preparing six assay sample solutions at a concentration of 125 µg mL^{-1} of Carvedilol, 45 µg mL^{-1} of Ivabradine oxalate (100% level w.r.t sample concentration) and injected into a UPLC system as per proposed method and calculated the percentage relative standard deviation (RSD) for assay results. Intermediate precision was established by preparing six assay sample solutions similar to precision on a different day by different analyst and then injected into a UPLC system as per proposed method. % RSD of assay results was calculated between two analyst values.

Robustness

The robustness of an analytical procedure measures its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. Method robustness was established by considering the variations in wavelength (285 ± 2 nm), flow rate (0.4 ± 0.1 mL min^{-1}), column oven temperature ($30°C \pm 5°C$) and a mobile phase ratio (50:50 v/v, 48:52 v/v and 52:48 v/v). Solution stability and filter interference were established.

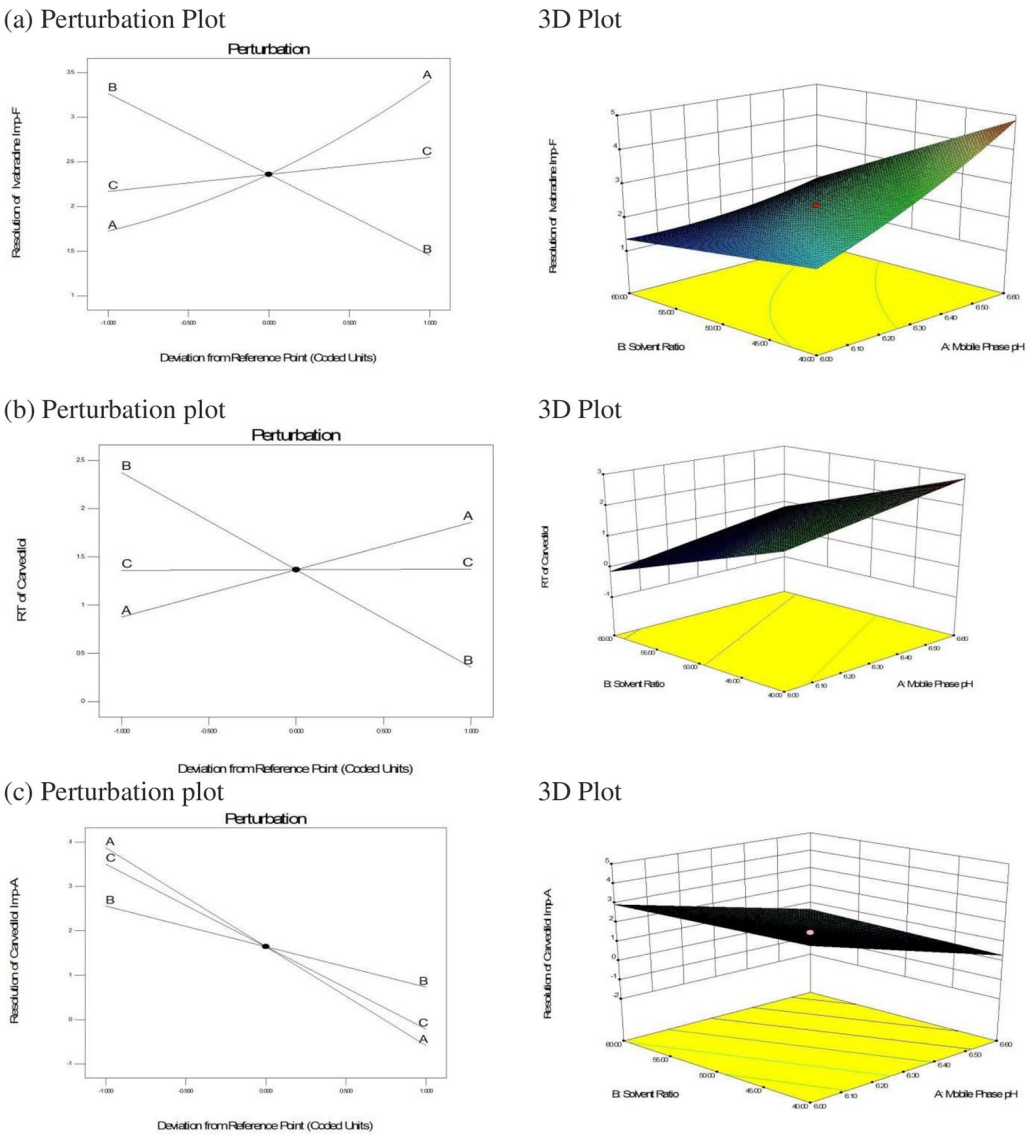

Figure 3. Effect of control variables (A) Mobile phase pH (B) Solvent ratio (C) Column temperature on response variables (a) Resolution between Ivabradine and Ivabradine impurity-F (b) Retention time of Carvedilol and (c) Resolution between Carvedilol and Carvedilol impurity-A.

Results and discussion

Method development and optimization

Wavelength maxima of Carvedilol were observed at 241.6 nm, 285.2 nm and 331.5 nm (Figure 2(b)). Wavelength maximum of Ivabradine was observed at 288.3 nm (Figure 2(a)). Detection wavelength of 285 nm was selected for simultaneous quantification of both Ivabradine and Carvedilol on the basis of common wavelength maxima from the individual compound spectral information. pH scouting studies were performed to select the suitable mobile phase buffer pH, to obtain shorter runtime with no interference of placebo and impurity peaks at the retention time of Carvedilol and Ivabradine. Mobile phases were prepared by mixing different pH buffers and acetonitrile in the ratio of 50:50 v/v. The results of pH scouting studies were given in Table 3. pH scouting studies disclose that the lower peak tailing, higher plate count with high resolution was achieved at mobile phase pH in the range of pH 6.0 to pH 7.0. In addition, Ivabradine impurity-F was well separated from Ivabradine peak at this pH range and impurity peak interference was observed at pH range of pH 2.0 to pH 5.0. Hence, pH of mobile phase buffer between 6.0 and 7.0 was selected for further chromatographic method optimization to achieve shorter run time with a lower retention time of Ivabradine and Carvedilol without any interference of placebo and degradation products.

Design-of-experiment studies

The design-of-experiment (DoE) study was performed to choose the robust and rugged operational chromatographic

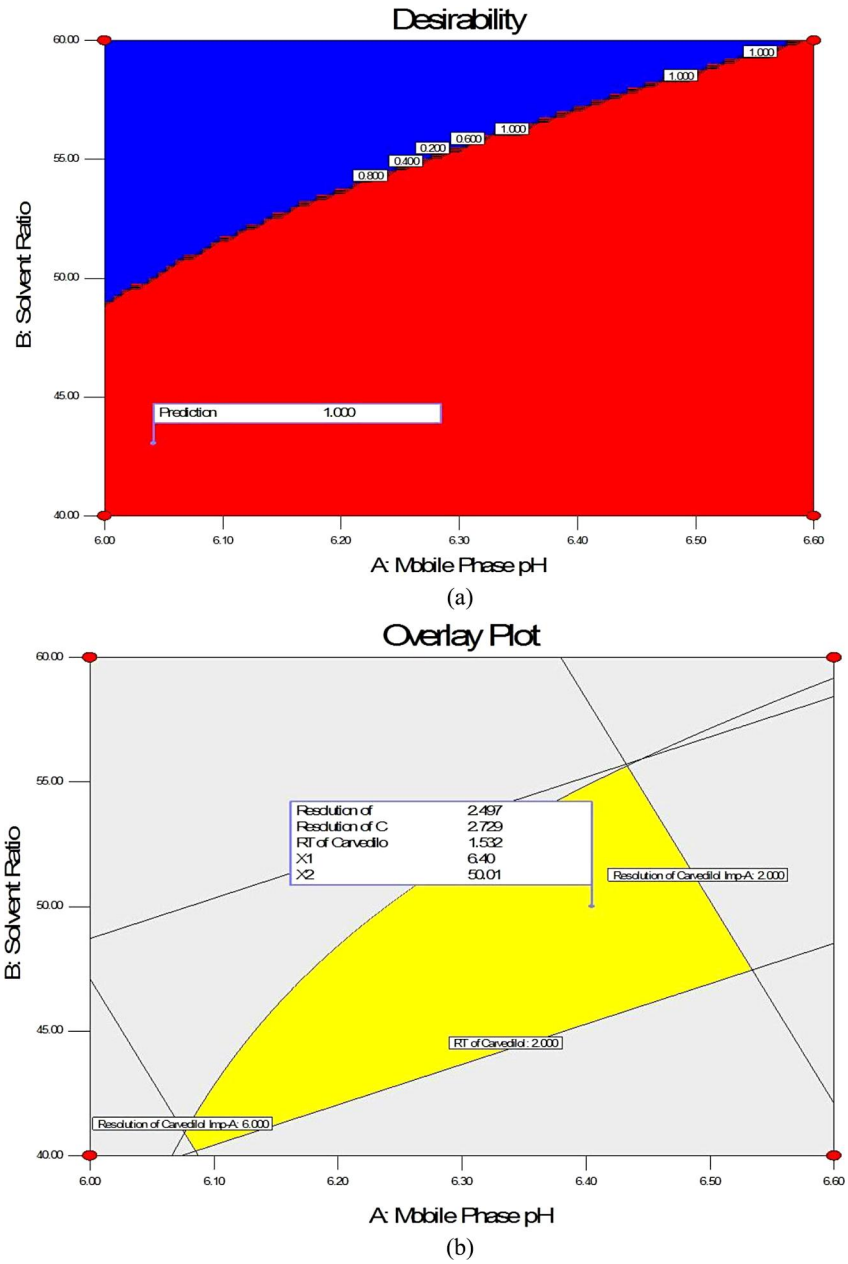

Figure 4. Schematic representation of desirability plot and design-space plot.

conditions within the design space. 0.5% v/v of triethylamine buffer (pH 6.3) was taken as a mobile phase buffer for DoE studies. DoE study was performed by considering the mobile phase composition, mobile phase pH and column temperature as control variables (Table 3). Resolution of Ivabradine impurity-F, resolution of Carvedilol impurity-A and retention time of Carvedilol were taken as response variables. Design-Expert 8.0 software was used for the study. The chosen design parameters are response surface as study type, central composite as design type and quadratic as the design model. Proposed ranges of mobile phase solvent composition 40 to 60%, mobile phase pH 6.0 to 6.6, and column temperature 30°C to 40°C were selected to execute the DoE studies. This data was fed into the design-expert software and it suggested fourteen experiments. All the DoE experiments were executed in UPLC and obtained results were tabulated in Table 4. The effect of control variables on response variables was graphically evaluated in Figure 3. The interpretation was derived from perturbation plot which describes the effect of method control variables on the response variables. Based on the design space plot (Figure 4), suitable chromatographic conditions were selected within the design space. Based on DoE experiments, mobile phase pH was selected at pH 6.4, column temperature was set at 30°C and acetonitrile composition in the mobile phase was set at 50% to achieve no interference of impurity peaks and shorter run time of two minutes.

Method validation

Specificity

No interference was observed with blank, placebo and impurities at the retention time of Carvedilol and Ivabradine peaks in the actual test sample. The system suitability test results observed during specificity and precision test were tabulated in Table 1. Acidic, basic, thermal, oxidation, water, humidity and photo-degradation samples were injected into UPLC system and extracted the chromatograms. No interference was observed with blank, placebo and impurities at the retention time of Carvedilol and Ivabradine in the forced degradation study samples. Carvedilol and Ivabradine peaks passed the peak purity test for all forced degradation study samples. The results were summarized in Table 1. The representative chromatograms of specificity were given in Figure 5 and Figure 6.

Linearity and range

Carvedilol and Ivabradine linearity solutions such as 20, 40, 80, 100, 120 and 160 percentage levels with respect to sample concentration were injected into UPLC system and chromatograms were recorded for linearity verification. The observed correlation coefficient value for both Carvedilol and Ivabradine was 0.9998. The regression line of analysis shows the linear relationship between concentration and area response of both Carvedilol and Ivabradine. Results of linearity and range were summarized in Table 2.

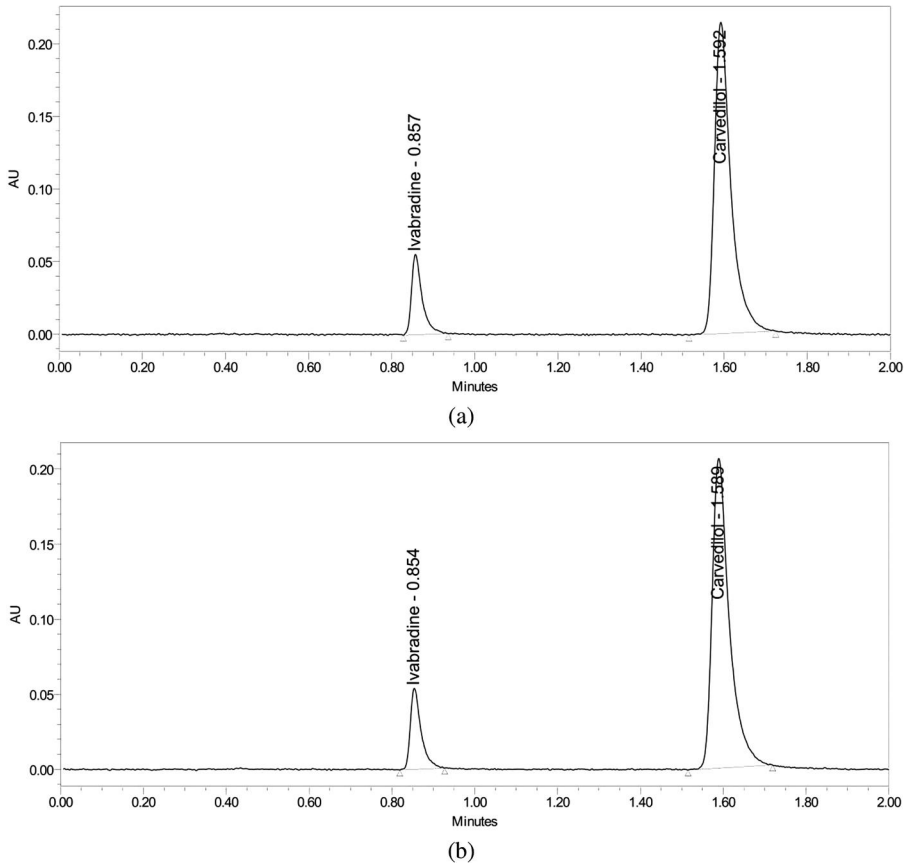

Figure 5. Representative chromatograms of (a) standard (b) sample.

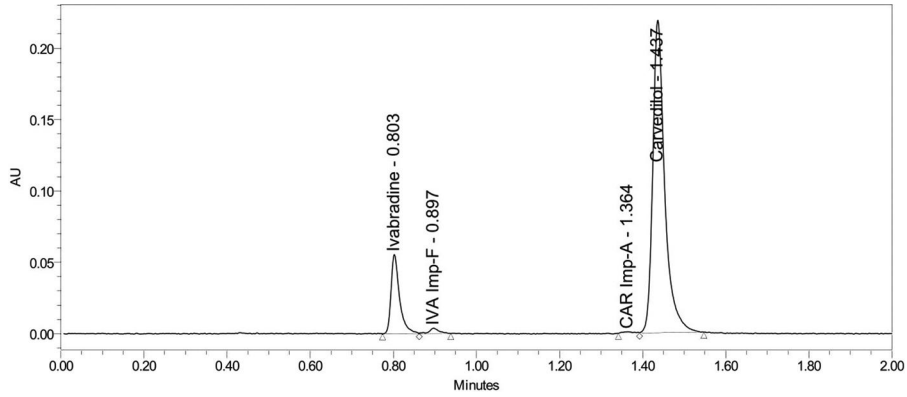

Figure 6. Representative chromatogram of impurity spiked sample.

Accuracy and precision

Carvedilol and Ivabradine drug substances were spiked to the placebo at 80, 100, 120, and 160 percentage levels with respect to the sample concentration and were analyzed by the proposed UPLC method. Recovery of Carvedilol and Ivabradine were observed in the range of 99.7% to 100.8% and 98.5% to 100.9% respectively. And all the individual results were within the range of 98.0 to 102.0% criteria for both Carvedilol and Ivabradine. The accuracy results were summarized in Table 5. The precision of the analytical method was proved by repeatability and intermediate precision. The percentage RSD results for repeatability were 0.32 and 0.16 for Ivabradine and Carvedilol, respectively (Table 5). The percentage RSD results between two analyst values were 0.88 and 0.77 for Ivabradine and Carvedilol, respectively. Since the percentage RSD of six assay results was not more than 2.0, the method was repeatable. The percentage RSD of two analyst's assay results was less than 2.0, hence, intermediate precision was acceptable.[27–29]

Robustness

Method robustness was established by considering the changes in wavelength, flow rate, column oven temperature, mobile phase buffer pH and mobile phase solvent ratio. Solution stability and filter interference were studied. Analytical solutions were stable for 24 hours. GHP and PVDF membrane syringe filters were evaluated for filter interference and no significant interference was observed. The results of robustness were tabulated in Table 2. Robustness test was passed as a variation from initial results to robustness test sample results was not more than 2.0%.[27–29]

Table 5. Accuracy and precision results.

	Ivabradine			Carvedilol		
Accuracy level	Theoretical conc.(μg mL^{-1})	Experimental conc.(μg mL^{-1})	% Recovery	Theoretical conc.(μg mL^{-1})	Experimental conc.(μg mL^{-1})	% Recovery
80% sample-1	35.895	35.692	99.4	99.607	100.386	100.8
80% sample-2	35.895	36.129	100.7	99.607	100.138	100.5
80% sample-3	35.895	36.219	100.9	99.607	99.820	100.2
100% sample-1	44.387	44.114	99.4	124.925	125.087	100.1
100% sample-2	44.387	44.721	100.8	124.925	124.591	99.7
100% sample-3	44.387	43.889	98.9	124.925	124.803	99.9
120% sample-1	53.228	52.787	99.2	150.903	150.317	99.6
120% sample-2	53.228	53.349	100.2	150.903	150.265	99.6
120% sample-3	53.228	52.437	98.5	150.903	150.426	99.7
160% sample-1	71.019	70.980	99.9	199.880	199.717	99.9
160% sample-2	71.019	71.004	100.0	199.880	200.491	100.3
160% sample-3	71.019	70.390	99.1	199.880	199.676	99.9
Average			99.8			100.0

Precision results				
	Ivabradine (%)		Carvedilol (%)	
Preparation	Repeatability	Intermediate precision	Repeatability	Intermediate Precision
1	98.6	100.2	100.0	99.8
2	98.9	98.3	99.9	99.0
3	98.7	99.0	100.0	98.4
4	98.5	97.4	100.0	101.0
5	99.0	100.1	99.9	101.1
6	98.1	100.2	99.6	100.7
Mean	98.6	99.2	99.9	100.0
%RSD	0.32	1.20	0.16	1.11
Mean between two analyst values		98.9		100
% RSD between two analyst values		0.88		0.77

Table 6. Comparison of selected analytical methods developed for assay of Carvedilol and Ivabradine.

S. No.	Column, elution process, mobile phase, flow rate	Sample linear range, detection	Run time	Intended use	Ref. no.
1	Inertsil ODS 250 × 4.6 mm, 5 µ column, 0.5% formic acid pH 7.0 and acetonitrile (65:35 v/v), 0.7 mL min^{-1}	4.2–31.6 µg mL^{-1}, HPLC-UV, spectroscopy	7 min	For estimation of Ivabradine (IVA) in tablets	[7]
2	48 cm length × 50 µ uncoated fused silica capillaries, 25 mM phosphate buffer pH 2.5 and 10 mM beta cyclodextrin	2.5–50 µg mL^{-1}, Capillary electrophoresis	12 min	For estimation of Carvedilol (CAR) enantiomers	[8]
3	Ace C18, 250 × 4.6 mm, 5 µ column, Isocratic, 20 mM phosphate buffer pH 7.0 and acetonitrile (65:35 v/v), 1.0 mL min^{-1}	10–250 ng mL^{-1}, HPLC-Flourescence	10 min	For estimation of Carvedilol release rate in biological sample	[9]
4	Hypersil BDS C18, 150 × 4.6 mm, 5 µ column, 0.01% triethylamine pH 3.3 and acetonitrile (70:30 v/v), 1.0 mL min^{-1}	41.4–62.1 µg mL^{-1}, HPLC-UV	10 min	For estimation of Carvedilol in drug product	[10]
5	Agilent C18, 250 × 4.6 mm, 5 µ column, 0.05 M KH_2PO_4 pH 2.5 and acetonitrile (60:40 v/v), 2.0 mL min^{-1}	10–200 µg mL^{-1}, HPLC-UV, TLC	5 min	For estimation of Carvedilol in drug product	[11]
6	Chiralcel ODH, 150 × 4.6 mm, 5 µ column, gradient, 0.05% TFA and 0.05% DEA in water and acetonitrile, 1.0 mL min^{-1}	CAR 25–200 µg mL^{-1}, HPLC-fluorescence	50 min	For estimation of Carvedilol and 5-hydroxy phenyl enantiomer in urine samples	[12]
7	Kinetex C18 150 × 4.6 mm, 5 µ column, 10 mM ammonium acetate pH 6.0 and methanol (50:50 v/v), 1.0 mL min^{-1}	70.69–131.29 µg mL^{-1}, HPLC-UV	5 min	For estimation of Ivabradine in tablets	[13]
8	Hypersil ODS C18 250 × 4.6 mm, 5 µ column, acetonitrile and phosphate buffer pH 3.0 (75:25 v/v), 1.0 mL min^{-1}	CAR 150–400 µg mL^{-1}, IVA 50–300 µg mL^{-1}, HPLC-UV	15 min	For estimation of Carvedilol and Ivabradine in tablets by HPLC	[14]
9	C8 100 × 2.1 mm, 1.8 µ UPLC column, 0.5% triethylamine pH 6.4 and acetonitrile (50:50 v/v), 0.4 mL min^{-1}	CAR 25.0–199.9 µg mL^{-1}, IVA 8.9–21.3 µg mL^{-1}, UPLC-UV	2 min	For estimation of Carvedilol and Ivabradine in tablets by UPLC	Present method

Comparison with reported methods

In the present study, an UPLC method was developed for simultaneous quantification of Carvedilol and Ivabradine in presence of degradation impurities in pharmaceutical finished dosage forms. The present method employs the shortest run time of two minutes for quantification of both the drugs compared to earlier reported methods which are having fifteen minutes.[14] The method was developed based on DoE approach and chromatographic conditions were selected from the design space. The developed method was stability-indicating as there was no interference in force degradation studies (Table 1). The method was validated as per ICH guidelines and the results of specificity, linearity, accuracy, precision, and robustness were found satisfactory. The reported methods were given for estimation of either Carvedilol or Ivabradine or in combination with other drugs in drug products. A detailed comparison of selected procedures with the present method was given in Table 6.

Conclusions

The UPLC method was developed based on the design-of-experiments approach for simultaneous, rapid quantification of Carvedilol and Ivabradine in the bulk and pharmaceutical drug products. The developed method was validated as per international council for harmonization guideline ICH Q2 (R1) validation of analytical procedures. The method was found to be simple, selective, accurate, precise and robust. The developed method was stability indicating as it was showing no interference of degradation products and placebo at the retention time of Carvedilol and Ivabradine. Due to the shorter run time of two minutes, this method provides faster analysis, more work throughput and reduces the cost of analysis due to the reduction in solvent consumption. Therefore, the developed method can be used for routine assay analysis of quality control samples and stability samples of bulk and finished pharmaceutical dosage forms.

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