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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CARVEDILOL AND IVABRADINE BY RP-HPLC METHOD

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ABSTRACT

This study deals with the development and validation of a stability-indicating RP-HPLC method for the quantitative estimation of Carvedilol and Ivabradine in bulk and pharmaceutical dosage preparations. Both agents are widely used in the treatment of cardiovascular diseases: Carvedilol is a non-selective beta-adrenergic antagonist with alpha-1 blocking activity, while Ivabradine inhibits selectively the If channel, causing the heart rate to go down. A precise analytical tool has to be developed-providing evidence of its accuracy, precision, specificity, and ruggedness-prior to the further quality control and stability studies. The method development was done by optimizing the chromatographic parameters such as mobile phase composition, flow rate, and detection wavelength to achieve adequate resolution and symmetrical peak morphology. The proposed method was subsequently validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantitation, and robustness according to ICH. The various forced degradation studies - thermal, oxidative, photolytic, acidic, and alkaline - were done in order to evaluate the capability of the method regarding separation between degradation products and analytes to provide stability indicating. The obtained results ensure that the method is appropriate to quantify both drugs in presence of degradation products for the quality control and stability study of pharmaceutical formulations.

KEYWORDS: Carvedilol, Ivabradine, Stability-indicating method, Forced degradation, Method validation, ICH guidelines.

INTRODUCTION

The quality, safety, and therapeutic efficacy of pharmaceutical products heavily rely on the use of reliable and well-established methods of analysis. Pharmaceutical analysis is essential to drug development and quality assurance in that it enables correct identification, quantification, and control of active pharmaceutical ingredients and related substances. To this end, precise analytical techniques are vital for ensuring product quality and compliance with regulatory requirements.

The high sensitivity, accuracy, and reproducibility of HPLC analyses have made it one of the most widely used analytical techniques in pharmaceutical analysis. It is thus widely employed for the routine analysis of drugs in bulk materials and finished dosage forms and for impurity determination and stability studies. Indeed, the ability of HPLC to effectively separate complex mixtures within a relatively short time frame makes it an indispensable tool in quality control laboratories.

Method development involves the systematic choice and optimization of chromatographic conditions to provide reliable analytical results that are reproducible. When official or pharmacopoeial methods are not available, the need for developing a new analytical methodology arises to facilitate routine quality control testing. The developed methods must be simple, inexpensive, and amenable to routine use in laboratories. A developed analytical method has to be validated to prove its suitability for the analyte under determination.

This is important in pharmaceutical analysis because method validation provides documented evidence that the analytical procedure will consistently yield results that are accurate and precise. The section of validation parameters includes accuracy, precision, linearity, specificity, robustness, and sensitivity. These parameters follow the guidelines laid down by the International Council for Harmonisation and ensure the reliability of the method and its applicability in pharmaceutical quality assurance.

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Thus, this study intends to develop and validate a simple, accurate, precise, and robust HPLC method for the quantitative estimation of the selected drug in a pharmaceutical dosage form in accordance with ICH guidelines.

MATERIALS AND METHODS

Drugs and Marketed Formulation: Carvedilol and Ivabradine working standards were obtained as gift samples from Akums Drugs & Pharmaceutical Ltd., New Delhi, India. The marketed formulation Ivabrad-C 6.25 was procured from the local market and manufactured by Lupin Ltd., labelled to contain Carvedilol (6.25 mg) and Ivabradine (5mg) per tablet.

Chemicals and Reagents: All chemicals and reagents used during the study were of analytical or HPLC grade. Methanol, Acetonitrile and Water (HPLC grade) were used for the preparation of mobile phase and standard solutions. Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH) and Hydrogen Peroxide (H_2O_2) of analytical reagent grade were employed for forced degradation studies. All solutions were prepared using freshly generated HPLC-grade water.

Instrumentation: The chromatographic analysis was performed using a JASCO HPLC system (Model PU-2080 Plus) equipped with Borwin software (version 1.50), a Rheodyne injector with a 20µL loop, and a UV-VIS detector (Model UV-2075). Separation was achieved on a C18 column (250 x 4.6 mm, 4.6 µm, Nucleosil, Germany). A UV-visible double beam spectrophotometer (Shimadzu, Model 1780) was used for preliminary studies. An electronic balance (Shimadzu, Model AY-120) was used for weighing, and a sonicator was used for sample preparation. Purified water was obtained from a PURELAB UHQ-II water purification system (Elga Lab). All glassware used was of calibrated Borosil or equivalent grade.

Chromatographic Conditions: Chromatographic separation was carried out under isocratic conditions using a reversed-phase C18 column (250 x 4.6 mm, 4.6 µm). The mobile phase consisted of acetonitrile and methanol in the ratio of 50:50 (v/v). Prior to use, the mobile phase was filtered through a 0.45 µm membrane filter and degassed. The flow rate was maintained at 1.0 mL/min, and detection was performed using a UV detector at the selected wavelength. The injection volume was 20 µL, and all analyses were performed at ambient temperature. Under these chromatographic conditions, satisfactory resolution with well-defined and symmetrical peaks was obtained for both Carvedilol and Ivabradine.

Method Development

Preliminary Studies: The procured drug samples of Carvedilol and Ivabradine were evaluated for physical appearance and identity. Identification of both drugs was confirmed by infrared (IR) spectral analysis. Solubility studies were conducted in various solvents, and both drugs were found to be sufficiently soluble in methanol. Based on solubility and compatibility, methanol was selected as the solvent for the preparation of standard and sample solutions.

Preparation of Standard Solutions: Standard stock solutions of Carvedilol and Ivabradine were prepared separately by dissolving 10mg of each drug in methanol and diluting to 10 mL to obtain a concentration of 1000 µg/mL. Working standard solutions containing 100 µg/mL of each drug were prepared by appropriate dilution with methanol.

Selection of Detection Wavelength: Appropriate dilutions of standard solutions were scanned in the UV range of 200-400 nm. The wavelength exhibiting adequate absorbance for both drugs was selected for HPLC detection.

Selection of Mobile Phase and Chromatographic Conditions: During method development, different organic modifiers were evaluated to achieve optimal chromatographic separation of Carvedilol and Ivabradine. Acetonitrile was selected as the organic modifier based on its compatibility with the analytes and the chromatographic system. Various combinations of acetonitrile and methanol were investigated. Initial trials using acetonitrile-methanol in the ratios of 20:80 (v/v) and 60:40 (v/v) did not provide satisfactory chromatographic performance in terms of peak shape and retention behaviour. Further optimization led to the selection of a mobile phase consisting of acetonitrile and methanol in the ratio of 50:50 (v/v). This mobile phase was selected for subsequent analysis, and the chromatographic conditions were applied consistently during method validation and sample analysis.

Preparation of Sample Solution (Tablet Formulation Analysis): Tablets each containing 6.25 mg of Carvedilol and 5 mg of Ivabradine was weighed and powdered. Powder equivalent to 13 mg of Carvedilol (10 mg of Ivabradine) was transferred to 10 ml volumetric flask and was diluted with methanol and volume made to 10 ml (1300 µg/ml of Carvedilol and 1000 µg/ml of Ivabradine) with methanol. Solution was filtered and further dilutions were made with mobile phase to get the final concentration of 26 µg/ml of Carvedilol and 20 µg/ml of Ivabradine. Solution was injected under optimized conditions.

Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2 (R1) guidelines.

Specificity: Specificity was established by comparing chromatograms of blank, standard and sample solutions. No interfering peaks were observed at the retention times of Carvedilol and Ivabradine, confirming the specificity of the method.

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System suitability: System suitability was evaluated by assessing parameters such as retention time, theoretical plates, asymmetric factor and resolution. The results were found to be within acceptable limits, indicating satisfactory system performance.

Linearity and Range: Linearity was demonstrated over the concentration range of 13-78 µg/mL for Carvedilol and 10-60 µg/mL for Ivabradine. Calibration curves showed good linear relationships between peak area and concentration with correlation coefficients close to unity. The range of the method was confirmed based on acceptable linearity, precision and accuracy within these concentration limits.

Precision: Precision was evaluated in terms of intra-day and inter-day precision by analyzing three different concentrations in triplicate. The %RSD values for both drugs were found to be within acceptable limits, indicating good repeatability and intermediate precision.

Accuracy: Accuracy was assessed by recovery studies using the standard addition method at 50%, 100% and 150% levels. The mean percentage recovery for both Carvedilol and Ivabradine was close to 100%, demonstrating the accuracy of the method.

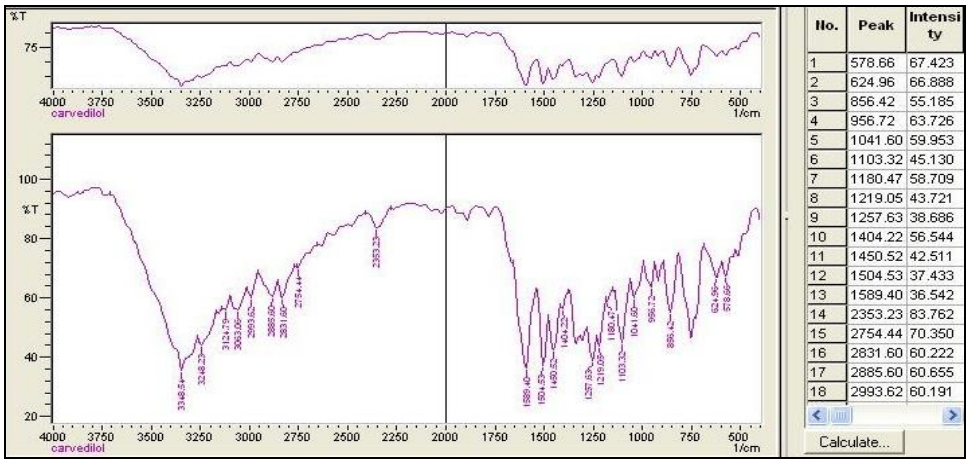
LOD & LOQ: The limit of detection (LOD) and limit of quantification (LOQ) for Carvedilol and Ivabradine were calculated in accordance with ICH Q2 (R1) guidelines using the standard deviation of the response (σ) and the slope of the calibration curve (S). LOD and LOQ were determined using the equations LOD = 3.3σ/S and LOQ = 10σ/S, respectively.

Robustness: The robustness of the method was evaluated by deliberately varying the chromatographic conditions i.e. altering mobile phase concentration by ± 2 ml and change in wavelength of detection by ± 1 nm and flow rate by ± 0.05 ml/min. At these altered conditions, the standard solutions were injected and effects were noted.

RESULTS AND DISCUSSION

Preliminary Study: The identity of Carvedilol and Ivabradine was confirmed by infrared (IR) spectroscopy. The recorded IR spectra of both drugs showed characteristic absorption bands corresponding to their functional groups, which were in good agreement with reported reference spectra. This confirmed the authenticity of the procured drug samples and their suitability for further analysis.

IR Spectrum of Carvedilol: The IR spectrum of Carvedilol exhibited characteristic absorption peaks corresponding to its functional groups, including aromatic C-H stretching, O-H stretching and C-N stretching vibrations. The observed peaks were consistent with standard reference data, confirming the chemical structure of Carvedilol.



No.	Peak	Intensity
1	578.66	67.423
2	624.96	66.888
3	856.42	55.185
4	956.72	63.726
5	1041.60	59.953
6	1103.32	45.130
7	1180.47	58.709
8	1219.05	43.721
9	1257.63	38.686
10	1404.22	56.544
11	1450.52	42.511
12	1504.53	37.433
13	1589.40	36.542
14	2353.23	83.762
15	2754.44	70.350
16	2831.60	60.222
17	2885.60	60.655
18	2993.62	60.191

Fig. 1: Carvedilol IR Spectrum.

IR Spectrum of Ivabradine: The IR spectrum of Ivabradine showed characteristic absorption bands attributable to functional groups such as aromatic C-H stretching, C=O stretching and C-N stretching vibrations. The spectral features were in agreement with reported literature values, confirming the identity of Ivabradine.

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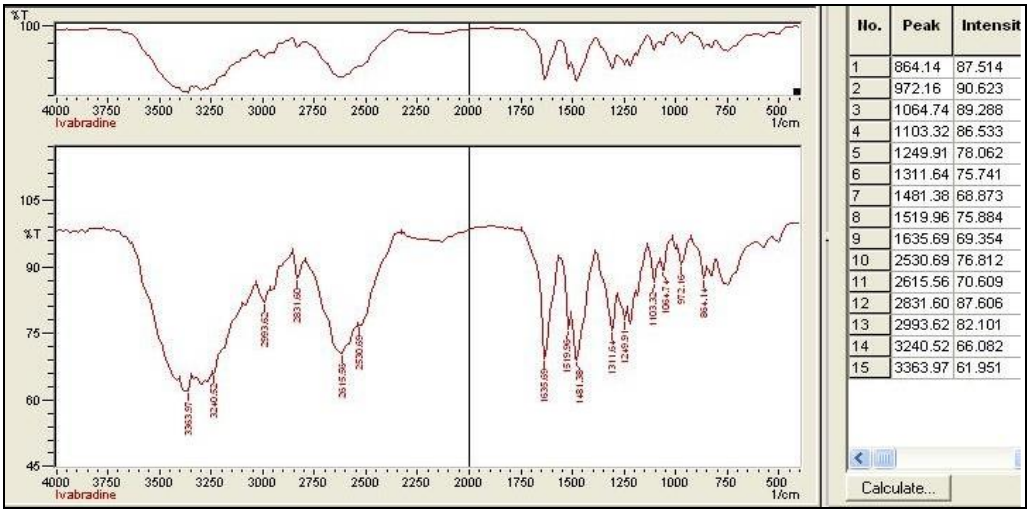

Fig. 2: Ivabradine IR Spectrum.

Solubility Analysis: Solubility analysis of Carvedilol and Ivabradine was carried out in different solvents to aid in method development. Carvedilol was found to be freely soluble in 0.1 N HCl, methanol and ethanol, slightly soluble in 0.1 N NaOH, and poorly soluble in water. Ivabradine was soluble in 0.1 N HCl, methanol and ethanol, slightly soluble in 0.1 N NaOH, and practically insoluble in water. Based on the solubility characteristics of both drugs and their compatibility with the chromatographic system, methanol was selected as the solvent for the preparation of standard and sample solutions throughout the study.

Optimization of Mobile Phase Composition: Under the optimized chromatographic conditions, the selected mobile phase enabled effective separation of Carvedilol and Ivabradine with well-defined and symmetrical peaks. Acceptable retention times and adequate resolution were obtained without interference from formulation excipients. These results confirm the suitability of the optimized mobile phase for simultaneous estimation and subsequent method validation.

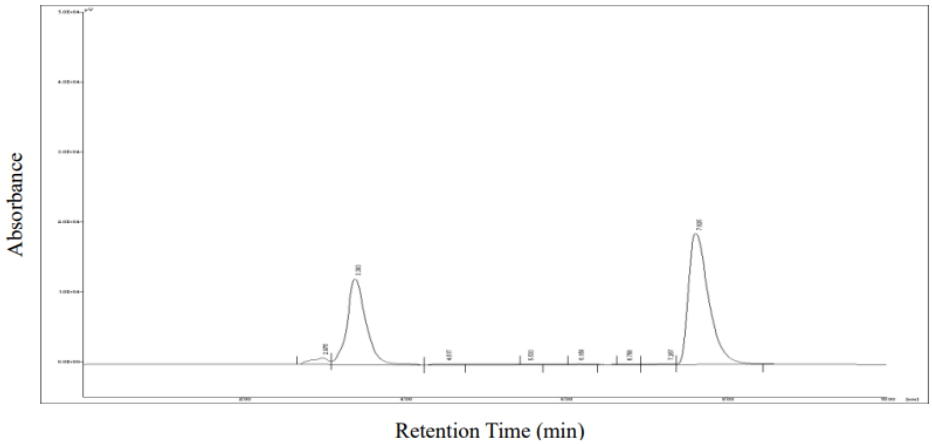

Fig.3: Optimized HPLC Chromatogram of Carvedilol and Ivabradine using Acetonitrile-Methanol (50:50 v/v).

Selection of Detection Wavelength: From the standard stock solution, further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 288 nm.

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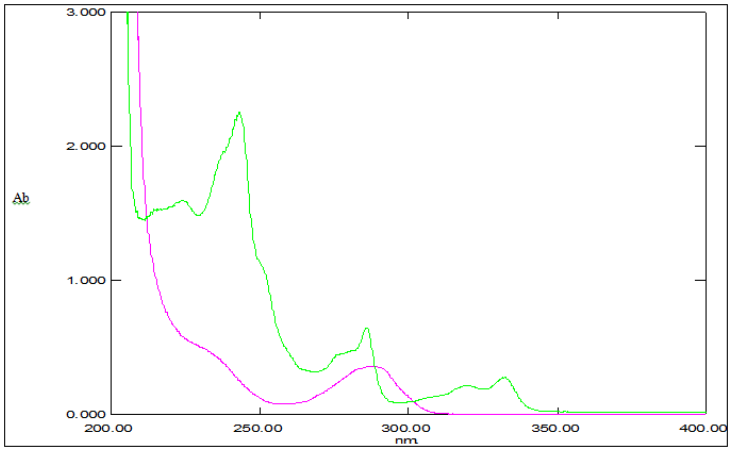

Fig.4: Overlay UV-VIS Spectra of A) Carvedilol (20 µg/ml, Green) and B) Ivabradine (20 µg/ml, Pink).

Validation of Analytical Method: The developed and optimized method was validated as per ICH Q2 (R1) guidelines. The method was validated in terms of specificity, system suitability, linearity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

a. Specificity: Specificity was checked by injecting blank and comparing the peaks observed in sample solution with standard solution. No interference was observed. Observed peaks in sample solution matched standard peak of Carvedilol and Ivabradine showed that, the method was specific.

b. System suitability: System suitability performance was evaluated by system suitability parameters such as retention time, theoretical plates, asymmetric factor and resolution and the method indicated good performance of the system.

Table 1: System suitability parameters for Carvedilol and Ivabradine.

Drugs	Concentration (µg/ml)	RT (min)	Area	Plates	Asymmetry	Rs*
Carvedilol	26	3.389 ± 0.137	1051396.253	2260.153	1.29	--
Ivabradine	20	7.609 ±0.249	807300.184	4999.609	1.70	7.19

* Resolution with respect to previous peak

c. Linearity: Linearity is the ability of the analytical method to obtain results that are directly proportional to concentration of the analyte in the sample. It was evaluated by determining six different concentrations of the standard working solutions in range of 13-78 µg/ml of Carvedilol and 10-60 µg/ml for Ivabradine. From the standard stock solution (1000 µg/ml) of Carvedilol and Ivabradine (1000 µg/ml), solution was prepared containing 100 µg/ml of Carvedilol and 100 µg/ml of Ivabradine, separately in methanol. These solutions appropriately diluted with mobile phase to get concentrations 13-78 µg/ml of Carvedilol and 10-60 µg/ml of Ivabradine. Each solution was injected on system and peak area was noted. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients.

Table 2: Linearity Study of Carvedilol.

Conc. µg/ml	Area 1	Area 2	Area 3	Area 4	Area 5	Average	SD	% RSD
13	463138.225	481702.527	471182.326	478213.042	472171.108	473281.446	7137.215	1.508
26	1051396.253	1054519.650	1071985.831	1044679.282	1044669.105	1053450.024	11213.482	1.064
39	1440548.820	1500552.867	1477608.738	1474049.729	1431938.187	1464939.668	28265.747	1.929
52	1984872.289	1999177.953	2022682.067	2028942.837	2061623.493	2019459.728	29520.066	1.462
65	2530586.593	2561087.759	2595562.225	2457638.660	2456253.995	2520225.846	62173.476	2.467
78	3098920.084	3030623.416	3040584.746	2977241.248	3039800.347	3037433.968	43201.900	1.422

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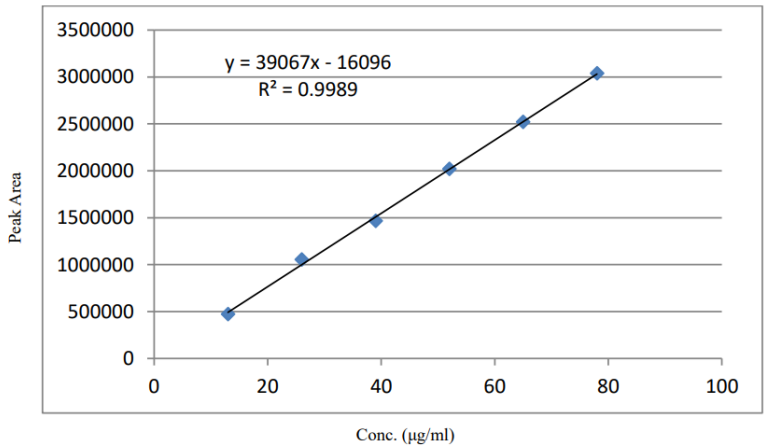

Fig. 5: Calibration Curve for Carvedilol.

Table 3: Linearity Study of Ivabradine.

Conc. µg/ml	Area 1	Area 2	Area 3	Area 4	Area 5	Average	SD	% RSD
10	402033.786	387852.575	395549.550	404301.975	400510.550	398049.687	6543.160	1.644
20	807300.184	814363.186	812548.547	785741.200	795932.400	803177.103	12108.351	1.508
30	1139943.650	1165201.928	1178232.292	1137926.475	1160550.450	1156370.959	17200.491	1.487
40	1564267.182	1513113.030	1550344.200	1530572.400	1514749.725	1534609.308	22360.490	1.457
50	1903904.794	1946439.421	1945415.120	1928090.250	1887518.265	1922273.570	25974.490	1.351
60	2300034.600	2346870.749	2337889.947	2300136.075	2301964.200	2317379.114	23055.503	0.995

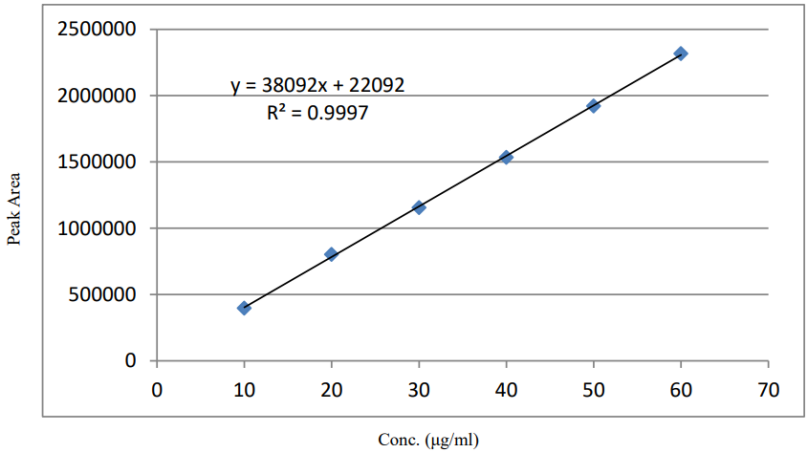

Fig. 6: Calibration Curve for Ivabradine.

d. Range: The range of an analytical method is the interval between the upper and lower levels of analyte that have been determined with precision, accuracy and linearity.
Carvedilol = 13-78 µg/ml
Ivabradine = 10-60 µg/ml

e. Precision: The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated.

Table 4: Intra-day Precision of Carvedilol.

Theoretical Concentration (µg/ml)	Area	Practical Concentration (µg/ml)	% Recovery	Avg % Recovery ± % RSD
26	1005985.831	26.162	100.624	100.184 ±0.405
	1000679.282	26.026	100.102	
	997873.6168	25.955	99.826	
52	2012202.27	51.918	99.843	99.942

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	2013966.456	51.964	99.930	±0.106
	2016490.122	52.028	100.054	
78	3024829.091	77.839	99.793	100.173 ±0.420
	3034191.788	78.078	100.100	
	3050164.223	78.487	100.625	

Table 5: Inter-day Precision of Carvedilol.

Concentration (µg/ml)	Area	Practical Concentration (µg/ml)	% Recovery	Avg % Recovery ± % RSD
20	782115.797	19.952	99.762	99.988 ± 0.256
	783440.658	19.987	99.936	
	785957.593	20.053	100.266	
40	1554896.043	40.240	100.599	100.341 ± 0.503
	1542099.809	39.904	99.759	
	1555903.549	40.266	100.665	
60	2299282.796	59.781	99.636	99.975 ± 0.471
	2318072.171	60.275	100.458	
	2299627.610	59.790	99.651	

Table 6: Intra-day Precision of Ivabradine.

Theoretical Concentration (µg/ml)	Area	Practical Concentration (µg/ml)	% Recovery	Avg % Recovery ± % RSD
26	1004979.845	26.137	100.525	100.236 ±0.265
	999678.603	26.001	100.003	
	1001469.641	26.047	100.180	
52	2024062.813	52.222	100.427	100.458 ±0.233
	2020268.664	52.125	100.240	
	2029727.626	52.367	100.706	
78	3038627.382	78.192	100.246	99.630 ±0.667
	3022515.46	77.779	99.717	
	2998415.866	77.163	98.926	

Table 7: Inter-day Precision of Ivabradine.

Concentration (µg/ml)	Area	Practical Concentration (µg/ml)	% Recovery	Avg % Recovery ± % RSD
20	783445.544	19.987	99.936	100.176 ± 0.263
	784958.850	20.027	100.135	
	787423.950	20.092	100.458	
40	1551339.184	40.146	100.365	100.199 ± 0.144
	1547657.114	40.049	100.124	
	1547400.600	40.043	100.107	
60	2299677.230	59.792	99.653	99.786 ± 0.385
	2295858.600	59.691	99.486	
	2312624.588	60.132	100.219	

f. Assay: Formulation analysis was carried out as mentioned under section formulation analysis. Procedure was repeated for six times. Sample solution was applied and area was recorded for each drug. Concentration and % purity were determined from linear equation.

Table 8: Assay of Tablet Formulation.

Sr. No.	Carvedilol			Ivabradine		
	Peak area	Amount recovered (µg/ml)	% Recovery	Peak area	Amount recovered (µg/ml)	% Recovery
1	1002973.116	26.085	100.328	781285.275	19.931	99.653
2	996411.498	25.917	99.682	786295.283	20.062	100.310
3	1001040.013	26.036	100.137	790949.025	20.184	100.921
4	998734.195	25.977	99.910	782690.790	19.967	99.837
5	999885.208	26.006	100.024	785602.350	20.044	100.219

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6	996668.890	25.924	99.707	785646.610	20.045	100.225
Mean	999285.487	25.991	99.965	785411.555	20.039	100.194
SD	2547.428	0.065	0.251	3345.593	0.088	0.439
% RSD	0.255	0.251	0.251	0.426	0.438	0.438

g. Accuracy: To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50%, 100% and 150%. Basic concentrations of sample chosen were 26 µg/ml of Carvedilol and 20 µg/ml of Ivabradine. These solutions were injected on HPLC system in triplicate to obtain the chromatograph. The drug concentrations of Carvedilol and Ivabradine were calculated by using linearity equations of Carvedilol and Ivabradine.

Table 9: Recovery Studies of Carvedilol.

Level	Conc. (ng/band)		Peak Area	Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.				
50 %	26	13	1506555.006	38.975	99.937	99.931 ± 0.186
			1509250.124	39.044	100.114	
			1503587.355	38.899	99.742	
100 %	26	26	2029460.135	52.360	100.693	100.103 ± 0.518
			2013205.017	51.944	99.893	
			2009752.495	51.856	99.723	
150 %	26	39	2534809.765	65.296	100.455	99.965 ± 0.425
			2516299.611	64.822	99.726	
			2515976.631	64.814	99.713	

Table 10: Recovery Studies of Ivabradine.

Level	Conc. (ng/band)		Peak Area	Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.				
50 %	20	10	1165169.925	30.008	100.028	100.215 ± 0.480
			1173554.890	30.228	100.762	
			1163191.811	29.956	99.855	
100 %	20	20	1552957.650	40.189	100.472	99.942 ± 0.474
			1542710.289	39.920	99.799	
			1539011.994	39.823	99.556	
150 %	20	30	1932754.275	50.159	100.318	100.225 ± 0.588
			1941208.815	50.381	100.762	
			1918957.725	49.797	99.594	

h. LOD & LOQ: The limit of detection (LOD) and limit of quantification (LOQ) for Carvedilol and Ivabradine were found to be within acceptable limits, indicating adequate sensitivity of the developed RP-HPLC method. The LOD values were 2.132 µg/mL for Carvedilol and 0.839 µg/mL for Ivabradine, while the LOQ values were 6.460 µg/mL and 2.542 µg/mL, respectively.

i. Robustness: The robustness of the method was evaluated by deliberately varying the chromatographic conditions i.e. altering mobile phase concentration by ± 2 ml and change in wavelength of detection by ± 1 nm and flow rate by ± 0.05 ml/min. At these altered conditions the standard solutions were injected and effects were noted and % RSD was calculated.

Table 11: Robustness Study.

	Carvedilol			Ivabradine		
	Wavelength (± 1nm)			Wavelength (± 1nm)		
	287	288	289	287	288	289
	1057444.178	1072887.153	1088193.401	805810.974	815238.225	812273.865
	1056650.850	1065628.704	1086878.154	802567.675	802288.704	833670.597
	1071218.642	1069245.333	1077364.737	807952.950	807239.205	824852.006
AVG	1061771.224	1069253.730	1084145.431	805443.866	808255.378	823598.823
STD DEV	8191.314	3629.232	5908.961	2711.342	6534.293	10753.273
% RSD	0.771	0.339	0.545	0.337	0.808	1.306

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RSD AVG	0.552			0.817		
	Flow Rate (± 0.05 ml/min)			**Flow Rate (± 0.05 ml/min)**		
	0.95	1	1.05	0.95	1	1.05
	1071961.790	1056162.647	1052616.858	801830.645	800719.650	802790.325
	1082997.807	1030822.653	1054094.894	811840.833	802614.768	800114.357
	1071529.707	1025343.617	1050746.559	801073.683	797126.785	805797.720
AVG	1075496.435	1037442.972	1052486.104	804915.054	800153.734	802900.801
STD DEV	6499.970	16441.552	1677.993	6009.831	2787.415	2843.292
% RSD	0.604	1.585	0.159	0.747	0.348	0.354
RSD AVG	0.783			0.483		
	MP Composition (± 2 ml)			**MP Composition (± 2 ml)**		
	48:52	**50:50**	**52:48**	**48:52**	**50:50**	**52:48**
	1072366.942	1058763.713	1086134.491	792725.400	803707.650	809262.675
	1069569.208	1057797.283	1038606.924	810797.943	797425.545	805450.835
	1088931.947	1068180.545	1055806.950	799657.040	811824.765	811768.050
AVG	1076956.032	1061580.513	1060182.789	801060.128	804319.320	808827.187
STD DEV	10465.359	5736.184	24064.047	9117.603	7219.071	3181.044
% RSD	0.972	0.540	2.270	1.138	0.898	0.393
RSD AVG	1.261			0.810		

FORCED DEGRADATION STUDIES

Stress degradation studies of bulk drug

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. Dry heat and photolytic degradation were carried out in solid state.

Alkaline hydrolysis: 1 ml working standard solution of Carvedilol (1000 µg/ml) was mixed with 1 ml of 1 N NaOH and 8 ml of methanol. The solution was kept for 24 Hr in dark place. This solution (100 µg/ml) was injected. Same procedure was repeated for Ivabradine.

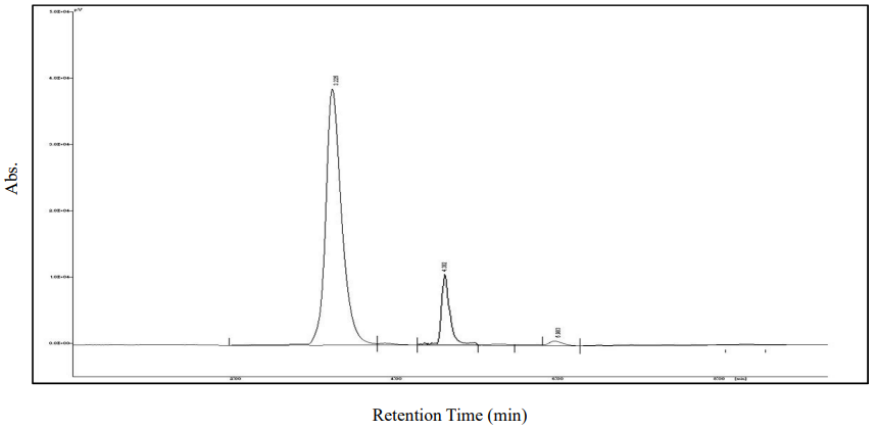

Fig.7: Chromatogram of Carvedilol (100 µg/ml) after alkaline hydrolysis.

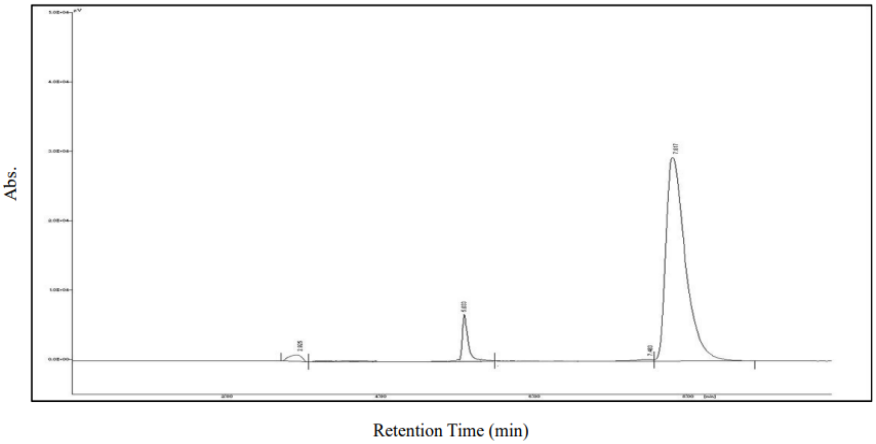

Fig. 8: Chromatogram of Ivabradine (100 µg/ml) after alkaline hydrolysis.

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Acidic hydrolysis: 1 ml working standard solution of Carvedilol (1000 µg/ml) was mixed with 1 ml of 1 N HCl and 8 ml of methanol. The solution was kept for 24 Hr in dark place. This solution (100 µg/ml) was injected. Same procedure was repeated for Ivabradine.

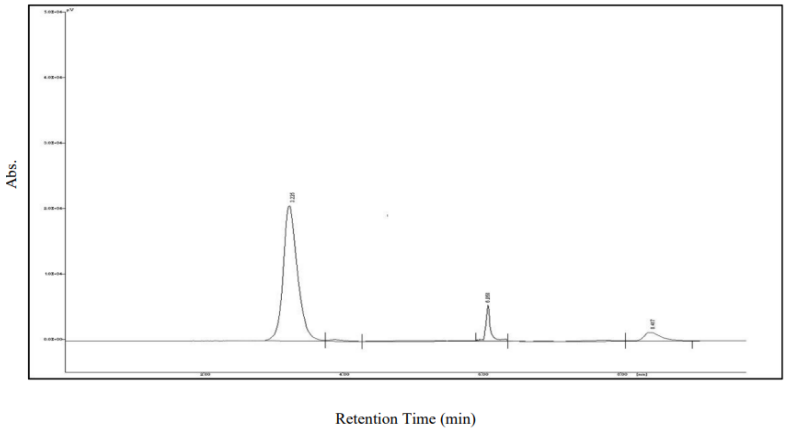

Fig. 9: Chromatogram of Carvedilol (10 µg/ml) after acid degradation.

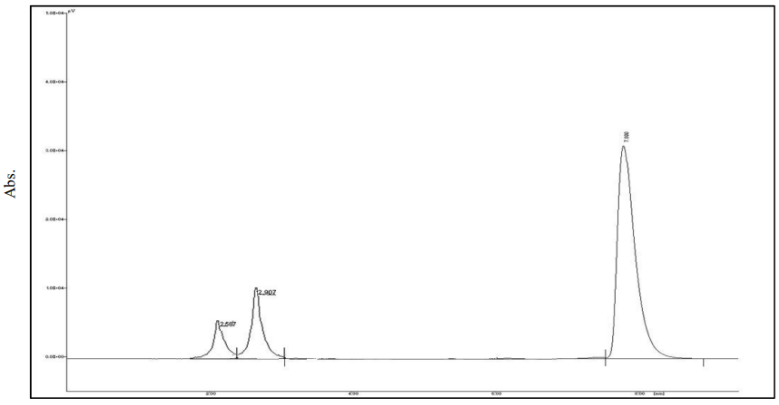

Fig. 10: Chromatogram of Ivabradine (10 µg/ml) after acid degradation.

Oxidation degradation: 1 ml working standard solution of Carvedilol (1000 µg/ml) was mixed with 1 ml of 30 % solution of H_2O_2 and 8 ml of methanol. The solution was kept for 24 Hr in dark place. This solution (100 µg/ml) was injected. Same procedure was repeated for Ivabradine.

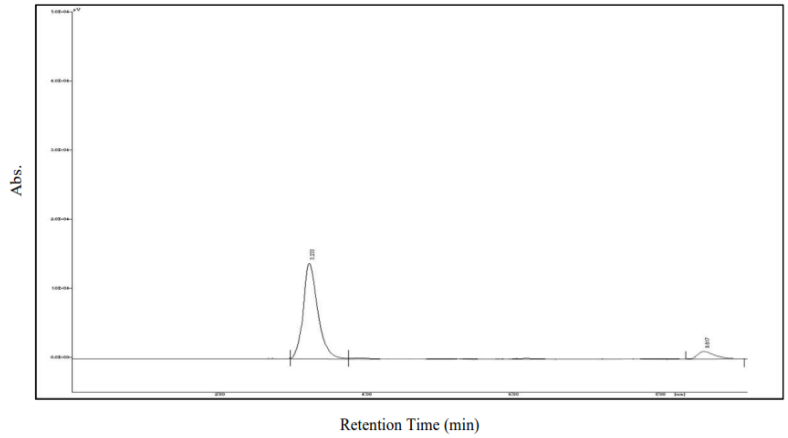

Fig. 11: Chromatogram of Carvedilol (10 µg/ml) after oxidation.

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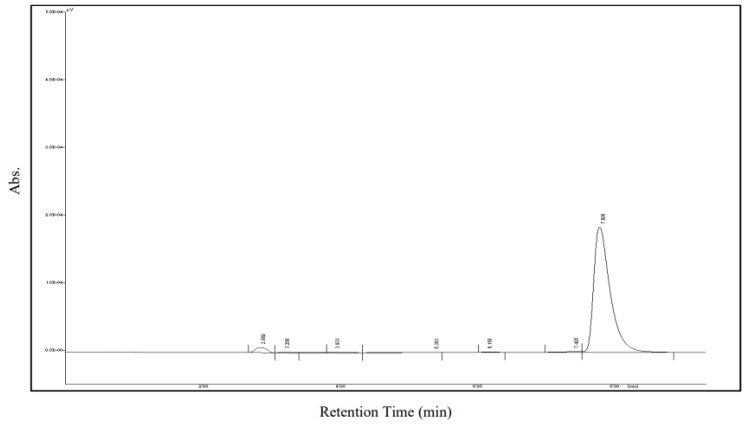

Fig. 12: Chromatogram of Ivabradine (10 µg/ml) after oxidation.

Degradation under dry heat: Dry heat studies were performed by keeping drug sample of Carvedilol & Ivabradine as individual in oven (100^0 C) for a period of 4 hour. Samples were withdrawn after 4 Hr, dissolved in methanol and further diluted to get 100 µg/ml as final concentration and were injected.

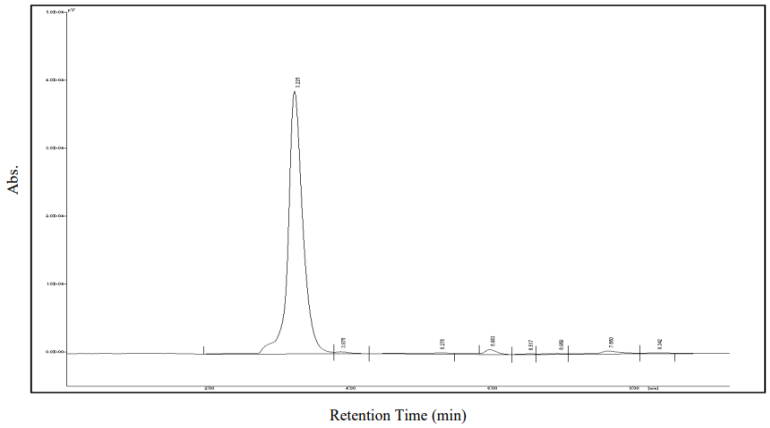

Fig. 13: Chromatogram of Carvedilol (100 µg/ml) after exposing to dry heat.

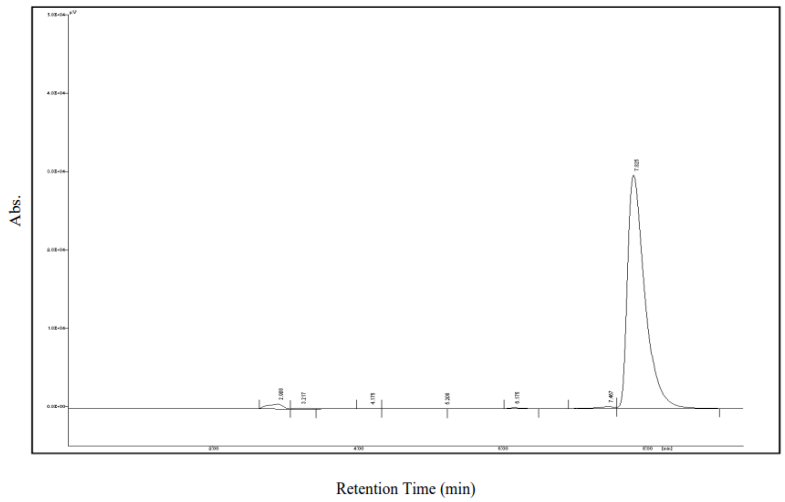

Fig. 14: Chromatogram of Ivabradine (100 µg/ml) after exposing to dry heat.

Photo-degradation studies: Photolytic studies were also carried out by exposure of drugs to UV light up to 200-watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. Sample was weighed, dissolved and diluted to get 100 µg/ml.

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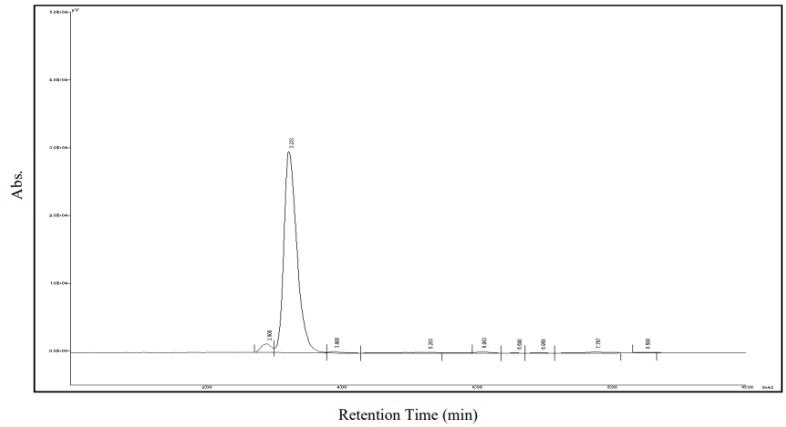

Fig. 15: Chromatogram of Carvedilol (100 µg/ml) after photo degradation.

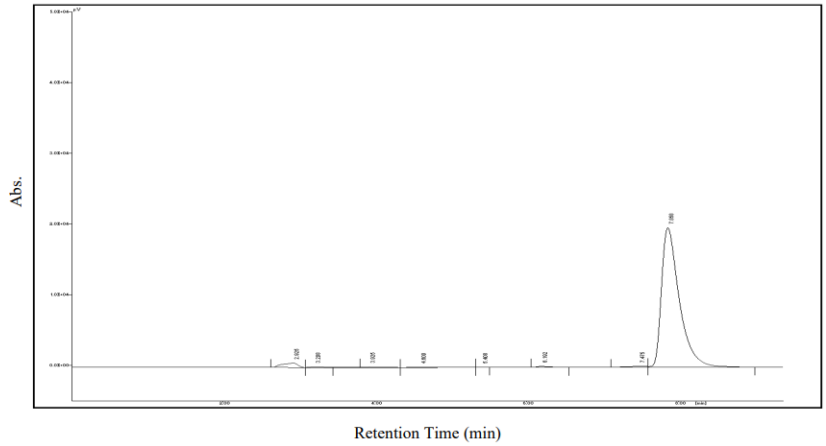

Fig. 16: Chromatogram of Ivabradine (100 µg/ml) after photo degradation.

Table 12: Summary of Stress Degradation Study of Carvedilol and Ivabradine.

Sr. No.	Stress Degradation Condition	Percent Recovery For Carvedilol (%)	Percent Recovery For Ivabradine (%)
1	Base (1 N NaOH, kept for 24 Hr)	89.53 (D1 - 4.382 D2 – 5.983)	92.87 (D1 - 5.983)
2	Acid (1 N HCl, Kept for 24 Hr)	91.32 (D2 – 6.058 D3 – 8.417)	87.14 (D2 - 2.587 D3 – 2.907)
3	H_2O_2 (30 %, kept for 24 Hr)	95.48 (D3 – 8.617)	98.54
4	Heat dry (1000C, 04 Hr)	98.28	99.46
5	Photo stability [UV, 200-watt hrs/square meter Florescence, 1.2 million Lux. Hrs]	99.14	98.82

CONCLUSION

A simple, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the estimation of Carvedilol and Ivabradine in bulk drug and pharmaceutical dosage forms. The method was validated in accordance with ICH Q2 (R1) guidelines and demonstrated satisfactory performance across all validation parameters. Forced degradation studies confirmed the stability-indicating capability of the method, with effective separation of the analytes from their degradation products. The developed method is suitable for routine quality control and stability studies in pharmaceutical analysis.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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