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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IVABRADINE AND METOPROLOL IN TABLET DOSAGE FORM

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Keywords:

Ivabradine, Metoprolol, Simultaneous estimation, RP-HPLC

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ABSTRACT: The objective of the method is to develop a rapid, simple, accurate, precise RP-HPLC method for the simultaneous estimation of Ivabradine and Metoprolol in tablet dosage form. The analytes were run through BDS C_{18} 150 × 46 mm, 5µm column, and detected at a wavelength of 260 nm using PDA 996 (photodiode array) detector. Mobile phase containing buffer 0.01N disodium hydrogen phosphate (3.5±1 pH) and acetonitrile in the ratio of 65:35 v/v was pumped through the column at a flow rate of 1.0ml/min and run time is about 6 min. The temperature was maintained at 30 °C. Retention times of ivabradine and metoprolol were found to be 2.38 min and 3.53 min. Percentage RSD of ivabradine and metoprolol was found to be 0.4 and 0.7, respectively. Percentage recovery of ivabradine and metoprolol were found to be 99.80% and 100.11%. LOD and LOQ values obtained from regression equations of ivabradine and metoprolol were found to be 0.03, 0.08, and 0.26, 0.79, respectively. The degradation studies were performed by applying different stress conditions like acidic, basic, thermal, photolytic, and results revealed that the method was a stable method. The developed method has good sensitivity, reproducibility, and specificity for the determination of ivabradine and metoprolol in bulk and its dosage form. The degradation studies were found to be stable and have the ability to separate degradation products in pharmaceutical dosage form and can be successfully applied for the simultaneous estimation of ivabradine and metoprolol in commercially available tablet dosage forms.

INTRODUCTION: Chemically, Ivabradine **Fig. 1** is 3-[3({(7s)-3,4-dimethoxy bicycle[4.2.0]octa-1,3, 5- trien- 7yl] methyl} (methyl) amino propyl]-7, 8 dimethoxy-2, 3, 4, 5 tetrahydro-H-3-benzazepin -2-one, having a molecular formula of $C_{27}H_{36}N_2O_5$ and molecular weight is 468.594 g/mol. It appears as a white crystalline powder, and pka value is 8.04. It acts as an anti-anginal agent by lowering the heart rate and has similar effects as that of β-blockers, but with low adverse effects, [1-2] compared to other drugs.

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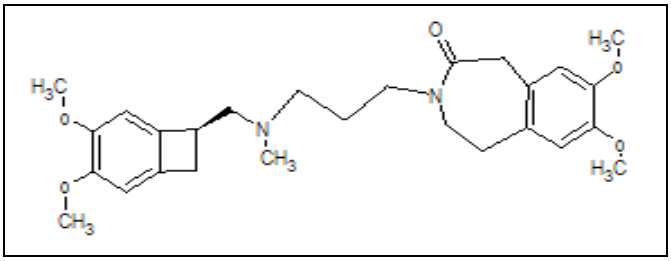

FIG. 1: IVABRADINE

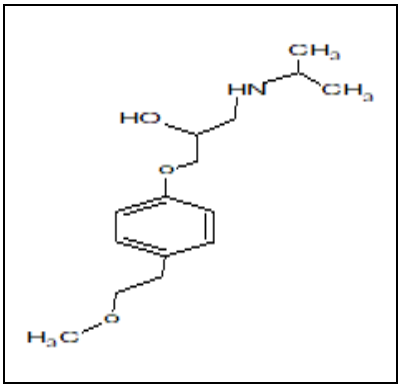

FIG. 2: METOPROLOL

Ivabradine is pulse bringing down medication for the symptomatic administration of stable angina pectorals and symptomatic perpetual heart. Depending upon the concentration, ivabradine acts by inhibiting pacemaker IF channels ("clever channels") in the heart. Metabolites of ivabradine are eliminated through feces matter and urine.

Metoprolol **Fig. 2** is chemically 1-4-(2-methoxy-ethyl) phenoxy]-3-[(propan-2-yl)amino]propan-2-ol, having molecular formula $C_{15}H_{25}NO_3$ and molecular weight is 267.3639 gm/mol. Metoprolol is a white crystalline powder and soluble in water, methanol and sparingly soluble in ethanol. Pka value is 9.36. It is a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI) [3], heart failure, angina pectoris, and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmia and prophylaxis for migraine headache [4].

The literature survey revealed that few UV [5, 6], RP-HPLC [7-11] methods for the individual estimation of ivabradine and metoprolol and a single RP-HPLC [12] method for simultaneous estimation of ivabradine and metoprolol are available. So, the present work focused on developing a rapid, specific, accurate and more economical validated RP-HPLC method for simultaneous estimation of ivabradine and metoprolol in a tablet dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: The standard drug samples of ivabradine and metoprolol were provided as gift samples from spectrum pharma research solutions Pvt. Ltd, Hyderabad. Marketed formulation of combination IVAMET, manufactured by Ajanta Pharma Ltd. Mumbai was purchased from a local pharmacy. HPLC grade water, acetonitrile, methanol and phosphate buffer, OPA (orthophosphoric acid) of AR (Analytical Reagent) grade were obtained from ranchem chemicals, Avantor Performance Materials India.

Instruments: The liquid chromatographic procedures were carried out by using WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, PDA 996 detector (photodiode array), autosampler and integrated with empower 2 software for data processing. The column used was a hypersil BDS C_{18} column and UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and quartz cells integrated UV win 6 software was used for measuring the absorbance of ivabradine and metoprolol solutions. Ultra-sonicator, the electronic balance of BVK enterprises, India, and denver pH-meter were used.

Diluent Used: A mixture of acetonitrile and water in the ratio of 50:50 was used as diluent. The mobile phase was prepared by mixing phosphate buffer and acetonitrile in the ratio of 65:35, and the pH was adjusted to 3.5 ± 1.

Preparation of Standard Stock Solution: An accurately weighed amount, 5 mg of ivabradine, and 25 mg of metoprolol were transferred into two 25 ml volumetric flasks separately. 15 ml of diluent was added to both the flasks and sonicated for 20 min. The volume was made up to the mark with diluent and labeled as standard stock solution 1 and 2 respectively, which contains 200 µg/ml of ivabradine and 1000 µg/ml of metoprolol, respectively.

Preparation of Standard Working Solution (100% Solution): From the above solution, 1ml was pipetted out and taken into a 10 ml volumetric flask, and final and the final volume was made up to mark with diluent to get a concentration of 20 µg/ml of ivabradine and 100 µg/ml of metoprolol.

Preparation of Sample Stock Solution: For the sample preparation, 20 tablets were accurately weighed, ground into a fine powder, and quantity equivalent to one tablet was transferred into a 50ml volumetric flask. 25 ml of diluent was added and sonicated for 25 min and the final volume was made up to mark with diluent and filtered by HPLC filters. The concentration of the solution was 100 µg/ml of ivabradine and 500 µg/ml of metoprolol.

Preparation of Sample Working Solution (100% solution): From the sample solution, 2ml was pipetted out to 10 ml volumetric flask, and the final volume was made up to mark with diluent, which contains 20µg/ml of ivabradine and 100µg/ml of metoprolol.

Chromatographic Conditions: The mobile phase of phosphate buffer and acetonitrile at the pH 3.5±1 was used for the ideal separation with different ratios and pumped through the different column (Symmetry C_{18} (4.6 × 250 mm, 5µm), Symmetry C_{18} (4.6 × 150 mm, 5µm), BDS C_{18} (4.6 × 150mm, 5µm) and BDS C_{18} (4.6 × 250 mm, 5µm) with the flow rate of 1ml/min.

The wavelength was set at 260 nm, and runtime was set at 10min and 6min for testing. Under the optimized chromatographic conditions, the peaks were eluted with good resolution, theoretical plates and acceptable tailing factor was given in **Fig. 3**.

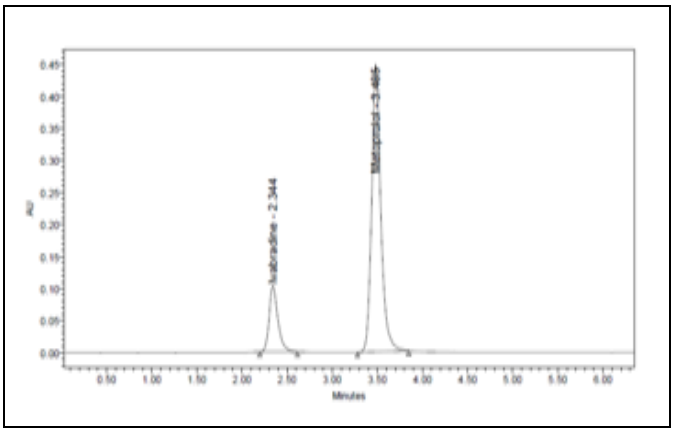

FIG. 3: OPTIMIZED CHROMATOGRAM

Forced Degradation Studies:

Acid Degradation: Acid degradation studies were determined by the addition of 1ml of 0.1N HCl to 1ml of sample and sonicated for 30 min at 60 °C. The final volume was made with diluent then the solution was injected into the chromatographic system. The chromatogram was recorded to assess the stability of the analyte.

Base Degradation: Base degradation studies were performed by addition of 1ml of 0.1N NaOH to 1ml of sample and sonicated for 30 min at 60 °C. The final volume was made with diluent then the solution was injected into the chromatographic system. The chromatogram was recorded to assess the stability of the analyte.

Peroxide Degradation: Peroxide studies were performed by the addition of 1ml of 0.1N H_2O_2 to 1ml of sample and sonicated for 30 min at 60 °C. The final volume was made with diluent and injected through a chromatographic system. The chromato-gram was recorded to assess the stability of the analyte.

Dry Heat Degradation (Thermal): The drug solutions of ivabradine and metoprolol were placed in an oven for 150 ºC for 1 h to study dry heat degradation. The resultant solutions were diluted to obtain 25 µg/ml of ivabradine and 250 µg/ml solution of metoprolol and injected into the chromatographic system. The chromatograms were recorded to assess the stability of the analyte.

Photolytic Degradation (UV): The photolytic stability of the drug was studied by exposing 25 µg/ml of ivabradine solution and 250 µg/ml of metoprolol solution to UV (Ultra-violet) light by keeping the beaker for 1day or 200 watt-hours/m^2 in photostability chamber. The resultant solutions were diluted with diluent to the required concentration and injected into the chromatographic system. The chromatograms were recorded to assess the stability of the analyte.

Humidity Degradation (Water): Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 ºC. The resultant solutions were diluted to obtain 25 µg/ml and 50 µg/ml and injected into the chromatographic system. The chromatograms were recorded to assess the stability of the sample.

RESULTS:

Method Validation:

Specificity: The specificity of the method was studied by injecting the mobile phase as blank, placebo, sample and standard solutions into the chromatographic system and checked for the interference with the retention times of ivabradine and metoprolol to establish specificity.

System Suitability: To ensure the performance of the chromatographic system, sample, and standard solutions were injected six times and evaluated for parameters such as plate count, theoretical plates, and tailing factor. The results of system suitability parameters were tabulated as in **Table 1**.

TABLE 1: SYSTEM SUITABILITY PARAMETERS FOR IVABRADINE AND METOPROLOL

S. no.	Ivabradine			Metoprolol			
Injection	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.347	3713	1.33	3.533	5253	1.18	6.1
2	2.352	3434	1.34	3.533	5395	1.16	6.3
3	2.355	3745	1.35	3.540	5594	1.16	6.5
4	2.361	3480	1.31	3.561	5417	1.14	6.6
5	2.369	3706	1.40	3.561	6398	1.16	6.9
6	2.380	3180	1.38	3.562	5859	1.15	6.7

RT: retention time and USP: United States of pharmacopeia

Precision: The precision of the analytical method was demonstrated by inter-day and intra-day studies (repeatability and reproducibility). The inter-day studies were carried out by determining the variation of the method by injecting six replicate analyte samples of the same concentration on two consecutive days. The intra-day studies were performed by injecting six test preparations into the chromatographic system as the assay. The repeatability and intermediate results were calculated in terms of percentage relative standard deviation (%RSD) and showed in **Table 2**.

TABLE 2: REPEATABILITY RESULTS OF IVABRADINE AND METOPROLOL

S. no.	Repeatability		Intermediate Precision	
	Area of Ivabradine	Area of Metoprolol	Area of Ivabradine	Area of Metoprolol
1.	786805	3593982	726164	3379609
2.	788772	3628332	737575	3362903
3.	790367	3554261	732739	3354165
4.	796139	3593964	734800	3359097
5.	791978	3606691	734311	3343825
6.	788665	3570702	740098	3351202
Mean	790454	3591322	734281	3358467
S.D	3286.2	26148.4	4758.6	12269.8
%RSD	0.4	0.7	0.6	0.4

%Relative standard deviation (%RSD)< 2%, mean= sum of the number of peak area/number of analyte and standard deviation

Linearity: Linearity was performed by plotting peak areas against their concentrations. Standard solutions in the range of 5-30 µg/ml of ivabradine and 25-150 µg/ml of metoprolol were taken and injected into the chromatographic system. The regression equations were y = 35904x+655.7 for ivabradine and y = 32557x+20569 for metoprolol (y= mx+c). Linearity plots were given in **Fig. 4**.

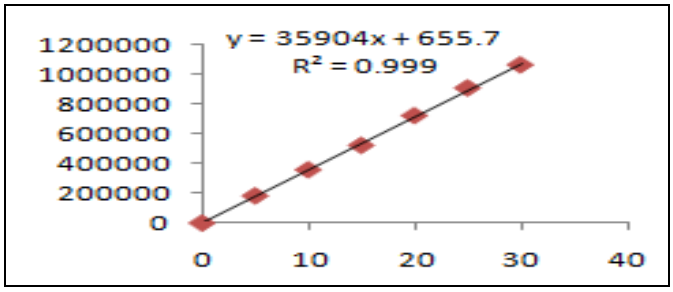

FIG. 4A: LINEARITY PLOT OF IVABRADINE

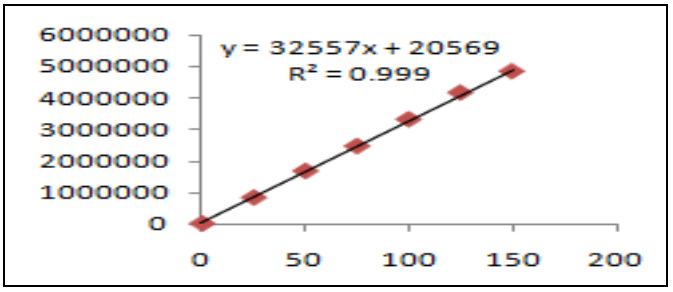

FIG. 4B: LINEARITY PLOT OF METOPROLOL

Accuracy: Recovery studies were carried out by the spiking method to reveal the accuracy of the developed method. The standard solutions with the concentration levels of 50%, 100%, 150% samples were injected thrice into the chromatographic system.

The mean percentage recoveries of ivabradine and metoprolol were calculated. The results of accuracy were tabulated in **Table 3**.

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) were identified based on the signal to noise ratio 3:1 and 10:1. These were analyzed, and values were measured by applying to linearity. Limit of detection* =3.3σ/s >2 and limit of quantification ** = 10σ/s>10, σ = standard deviation of response and s= slope of the regression line.

TABLE 3: ACCURACY DATA OF IVABRADINE AND METOPROLOL

	% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	
	50%	10	9.94	99.44	
		10	10.02	100.24	
		10	9.92	99.18	
	100%	20	19.85	99.26	
Ivabradine		20	20.08	100.38	99.80%
		20	19.97	99.87	
	150%	30	30.40	101.33	
		30	30.06	100.19	
		30	29.48	98.28	
	50%	50	50.40	100.80	
		50	49.65	99.30	
		50	50.21	100.42	
	100%	100	99.95	99.95	
Metoprolol		100	100.57	100.57	100.11%
		100	100.05	100.05	
	150%	50	150.80	100.53	
		50	149.65	99.77	
		50	149.43	99.62	

* Mean % recovery = sum of %recoveries / n, where n = number of samples (6)

Robustness: The robustness of the method was studied by making small changes in chromatographic conditions like flow rate (±0.1ml/min), mobile phase ratio (±10B:10A) and temperature (±5°C). The samples were injected in a duplicate manner, and the percentage RSD was calculated.

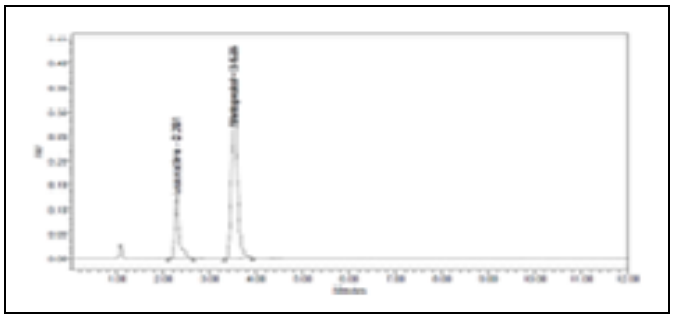

FIG. 5A: CHROMATOGRAM OF ACID STUDIES

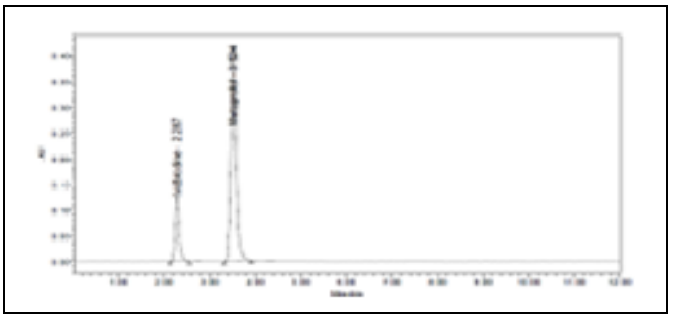

FIG. 5B: CHROMATOGRAM OF BASE STUDIES

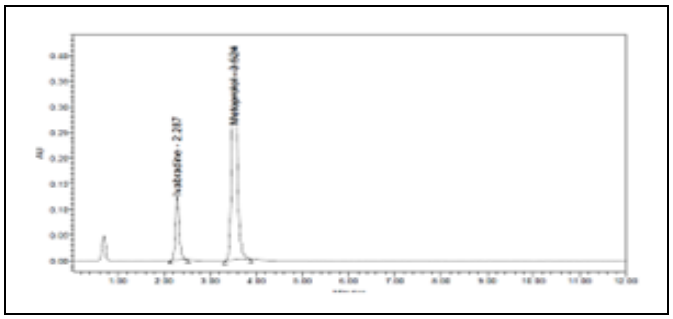

FIG. 5C: CHROMATOGRAM OF PEROXIDE STUDY

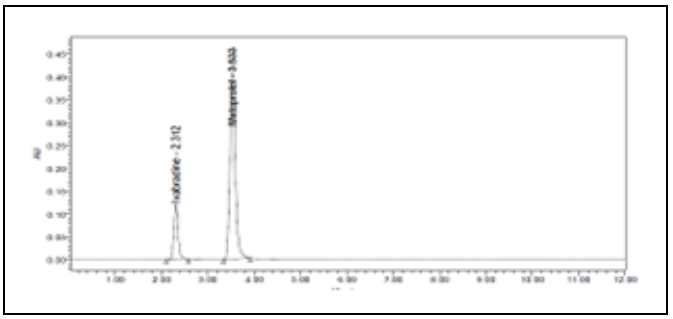

FIG. 5D: CHROMATOGRAM OF WATER STUDY

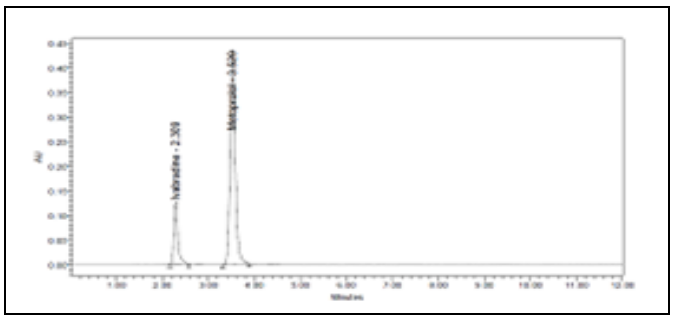

FIG. 5E: CHROMATOGRAM OF THERMAL STUDY

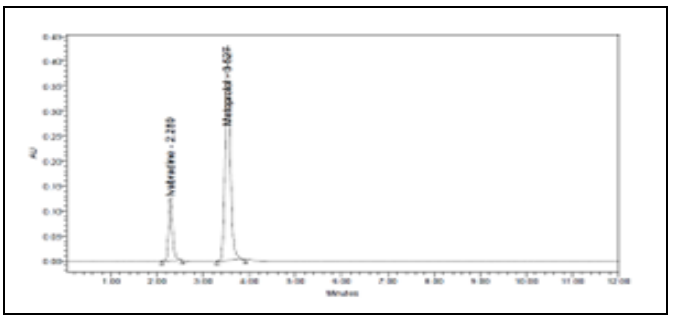

FIG. 5F: CHROMATOGRAM OF UV STUDY

Forced Degradation Studies: Stability indicating methods measure the active ingredient without inference from the degradation process. These studies were concluded by applying the stress to the analyte like acid, base, peroxide, UV, water, and thermal. The degradation data was given in **Table 4,** and chromatograms were shown in **Fig. 5.**

TABLE 4: RESULTS OF FORCED DEGRADATION STUDIES FOR IVABRADINE AND METOPROLOL

	Mode of degradation	Ivabradine				
		Area	% Recovered	% Degraded	Purity Angle	Purity Threshold
Ivabradine	Undegraded sample	790454	99.71	-	-	-
	Acid	757527	95.55	4.16	0.175	0.283
	Base	752284	94.89	4.82	0.167	0.280
	Peroxide	763899	96.36	3.35	0.168	0.279
	Thermal	769214	97.03	2.68	0.133	0.278
	Photolytic	778945	98.25	1.46	0.167	0.282
	Humidity	785082	98.25	0.76	0.151	0.281
Metoprolol	Undegraded Sample	3591322	99.74	-	-	-
	Acid	3402180	94.49	5.25	0.115	0.305
	Base	3419916	94.98	4.76	0.115	0.309
	Peroxide	3456436	96.00	3.74	0.115	0.306
	Thermal	3509426	97.47	2.27	0.116	0.303
	Photolytic	3529271	98.02	1.72	0.122	0.306
	Humidity	3562656	98.95	0.79	0.122	0.305

DISCUSSION: The method has been developed after performing several trails by changing columns (Symmetry C18 (4.6 × 250 mm, 5 µm), Symmetry C18 (4.6 × 150 mm, 5 µm), BDS C18 (4.6 × 150 mm, 5 µm) and BDS C18 (4.6 × 250mm, 5µm), the run time (6 min and 10 min) mobile phase ratios (buffer: acetonitrile pH 3.5 ± 1), temperature at 30 °C and injection volume about 10 µl/ml. The wavelength selected was 260 nm. All the peaks were eluted with good shape and satisfying system suitability parameters. The retention times of ivabradine and metoprolol were found to be 2.38 min and 3.53min in an optimized method. The precision of the method based on % RSD was found to be 0.4 for ivabradine and 0.7 metoprolol. The % RSD was within limits (<2), thus the method was precise for ivabradine and metoprolol. Linearity range was obtained by regression equations y = 35904x+655.7 for ivabradine and y = 32557x+20569 for metoprolol (y = mx + c). The accuracy was measured by calculating %recovery and was found to be 99.80% and 100.11% for ivabradine and metoprolol, respectively.

LOD, LOQ values obtained from the regression lines of Ivabradine and metoprolol were 0.03, 0.08, and 0.26, 0.79, respectively. Retention time and run time were decreased for ivabradine and metoprolol. In all the stress conditions of forced degradation studies, there was no significant degradation of the drug substance was observed, and in each condition, the purity threshold value was found to be greater than the purity angle value, and no purity flags were observed. This implies that there was no interference of degradants with that of analyte peaks, and the developed method was a stable method. The developed method was more economical than the available methods and can be used for the simultaneous estimation of ivabradine and metoprolol in the combined dosage form. The future plans of the studies involve accelerating the stress conditions like treating the drugs with high strength acid and alkali to get degradants and establishing the structures using hyphenated analytical techniques, which further provides better quality control for pharmaceutical industries.

CONCLUSION: The reported RP-HPLC method was proved to be simple, accurate, precise and more economical than the earlier reported method for the simultaneous estimation of ivabradine and metoprolol in tablet dosage forms. The retention times of ivabradine and metoprolol were found to be 2.38 min and 3.53 min. Degradation studies revealed that the developed method was stable. The chemicals utilized in the proposed method are economical and readily available. Hence, this developed RP-HPLC method is specific and simple. So, this method can be conveniently used for the simultaneous estimation of ivabradine and metoprolol in the tablet dosage form.

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CONFLICTS OF INTEREST: This study was unfunded. There were no conflicts of interest.

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