Research Article

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Stability indicating RP-HPLC method development and validation for estimation of ivabradine and metoprolol in pharmaceutical dosage form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivabradine and Metoprolol in tablet dosage form. Chromatogram was run through Ascentis C18 150mm x 4.6 mm, 3.6µm. Mobile phase containing 0.01N KH_2PO_4: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.8 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0nm. Retention time of Ivabradine and Metoprolol were found to be 2.181min and 2.669min. %RSD of the Ivabradine and Metoprolol were and found to be 0.6 and 0.3 respectively. %Recovery was obtained as 100.37% and 99.54% for Ivabradine and Metoprolol respectively. LOD, LOQ values obtained from regression equations of Ivabradine and Metoprolol were 0.11, 0.33 and 0.39, 1.19 respectively. Regression equation of Ivabradine is y = 5200.x + 310.0 and y = 9807.x + 6086 of Metoprolol. The method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Ivabradine, Metoprolol, RP-HPLC

INTRODUCTION

Ivabradine is a novel pulse bringing down medication for the symptomatic administration of stable angina pectoralis and symptomatic perpetual heart disappointment. Ivabradine acts by specifically hindering the "amusing" channel pacemaker current (If) in the sinoatrial hub in a portion subordinate design, bringing about a lower pulse and in this manner more blood to stream to the myocardium. Despite the fact that non-dihydropyridine calcium channel blockers and beta blockers additionally adequately bring down pulse, they show antagonistic occasions because of their negative ionotropic impacts [1].

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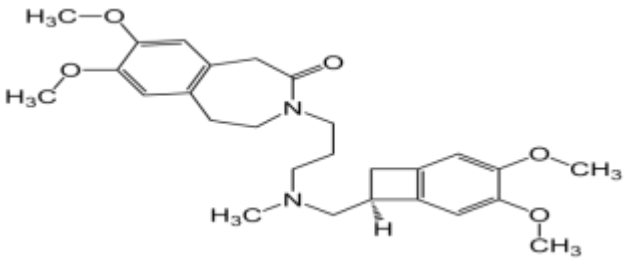

Fig.1: Chemical structure of Ivabradine

Metoprolol is a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. At low doses, metoprolol selectively blocks cardiac β1-adrenergic receptors with little activity against β2-adrenergic receptors of the lungs and vascular smooth muscle [2].

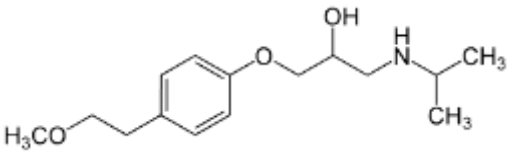

Fig. 2: Chemical structure of Metoprolol

The broad writing study uncovered that RP-HPLC [3-6] and UV spectrophotometric techniques [7] and HPLC strategy were accessible for the assurance of Ivabradine and Metoprolol separately or in mix with different medications. The investigation was in this manner performed with a plan to build up a straightforward, economic, sensitive, quick, exact, and stability representing RP-HPLC technique for the assurance of Ivabradine and Metoprolol in joined tablet measurements shape.

MATERIALS AND METHODS

Samples of Ivabradine, and Metoprolol are obtained from Spectrum Pharma labs, Hyderabad, India. HPLC grade distilled water, acetonitrile, methanol and AR grade Potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid, ortho-tri ethyl amine were obtained from Rankem. Waters HPLC 2695 system equipped with PDA detector with Empower 2 Software.

Preparation of standard stock solutions

Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml Ivabradine of and 100µg/ml of Metoprolol)

Preparation of sample stock solutions

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ivabradine and 100µg/ml of Metoprolol)

Preparation of diluent

Acetonitrile and water taken in the ratio of 50:50v/v

Preparation of buffer

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. orthophosphoric acid solution.

Method development

Initially, reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which drugs did not respond properly, and the resolution was also poor. The organic content of the mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of the mobile phase becomes an important factor. Ascentis C18 150mm x 4.6 mm, 3.6µm with an isocratic mobile phase composed of 0.01N Phosphate buffer and Acetonitrile mixed in the ratio of 60:40v/v at a flow rate of 0.8 ml/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 260 nm. The retention times were found to about 2.181min and 2.269min for ivabradine and metoprolol. The method was validated in terms of accuracy, precision and linearity as per ICH guidelines.

Method Validation

For Ivabradine and Metoprolol

System Suitability

The system suitability parameters were determined by preparing standard solutions of ivabradine 20 µg/ml and metoprolol 100 µg/ml. The solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%. The results were shown in **Table 1**.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. There should not find interfering peaks in the blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

By appropriate aliquots of the standard ivabradine and metoprolol prepared six working solutions ranging between 5-30µg/mL& 25-150µg/. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficient on curves for ivabradine and metoprolol. The results were shown in **Table 2**.

Accuracy

Accuracy was carried out by % recovery studies of ivabradine and metoprolol at three different concentration levels (50%, 100%, and 150%). Percentage recovery was calculated from the amount added and the amount recovered. The percentage recovery was within the acceptance criteria, this indicates the accuracy of the method. (Acceptance criteria: % recovery between 98 to 102). The results were shown in **Table 3**.

Precision

The repeatability of the method was verified by calculating the % RSD of six replicate injections of 100% concentration (20µg/ml of ivabradine and 100 µg/ml of metoprolol) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were shown in **Table 4**.

Robustness

Robustness of the method were verified by altering the chromatographic conditions like flow rate, mobile phase ratio and temperature are made, but there were no recognized change in the result and all are within range as per ICH guidelines. Robustness conditions like flow minus (0.7 ml/min), flow plus (0.9ml/min), 65:35mobile phase minus 55:45 mobile phase plus, temperature minus (25 °C) and temperature plus (35 °C) were maintained and samples were injected in duplicate manner. System suitability parameter was passed. % RSD was within the limit. The result was shown in **Table 5**.

DEGRADATION STUDIES

Acid degradation

To 1 ml of stock solution ivabradine and metoprolol), 1ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60 °C. The resultant solution was diluted to obtain 20µg/ml and 100µg/ml solutions and 10.0µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Oxidative Degradation

To 1 ml of stock solution of ivabradine and metoprolol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60 °C. For HPLC study, the resultant solution was diluted to obtain 20µg/ml and 100µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation

To 1 ml of stock solution ivabradine and metoprolol,, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 20µg/ml and 100µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Thermal Degradation

The standard drug solution was placed in oven at 105 °C for 6 hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 20µg/ml and 100µg/ml solution and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Degradation

The photochemical stability of the drug was also studied by exposing the 200µg/ml and 1000µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hrs/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml and 100µg/ml solutions and 10.0µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, and mobile phase containing 0.01N Potassium dihyrogen Ortho phosphate buffer and acetonitrile taken in the ratio of 60:40v/v was selected as mobile phase because of better resolution more no. of Theoretical plates and symmetric peaks. Ivabradine and metoprolol were found to show appreciable absorbance at 260nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of ivabradine and Metoprolol.

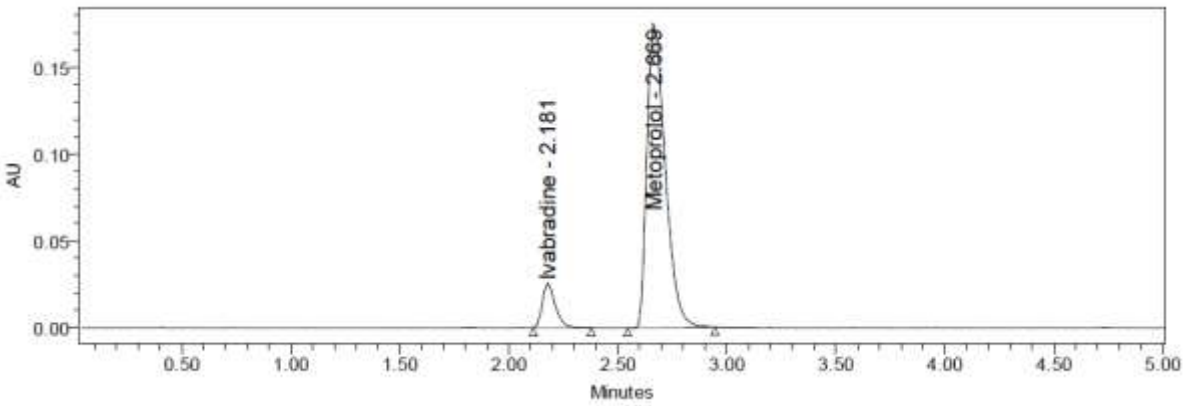

Fig.3: Optimized chromatogram of Ivabradine and Metoprolol

System Suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Table 1: System suitability parameters

Parameter	IVABRADINE	METOPROLOL
Retention time (min)	2.181	2.668
Theoretical plates (N)	6776	4734
Tailing factor (T)	1.34	1.45
Resolution		3.7

Linearity

Concentration range of 5-30µg/ml for ivabradine and 25-150µg/ml of metoprolol were found to be linear with correlation coefficients 0.999 were within limits. The result was shown in **Fig. 4** and **5**

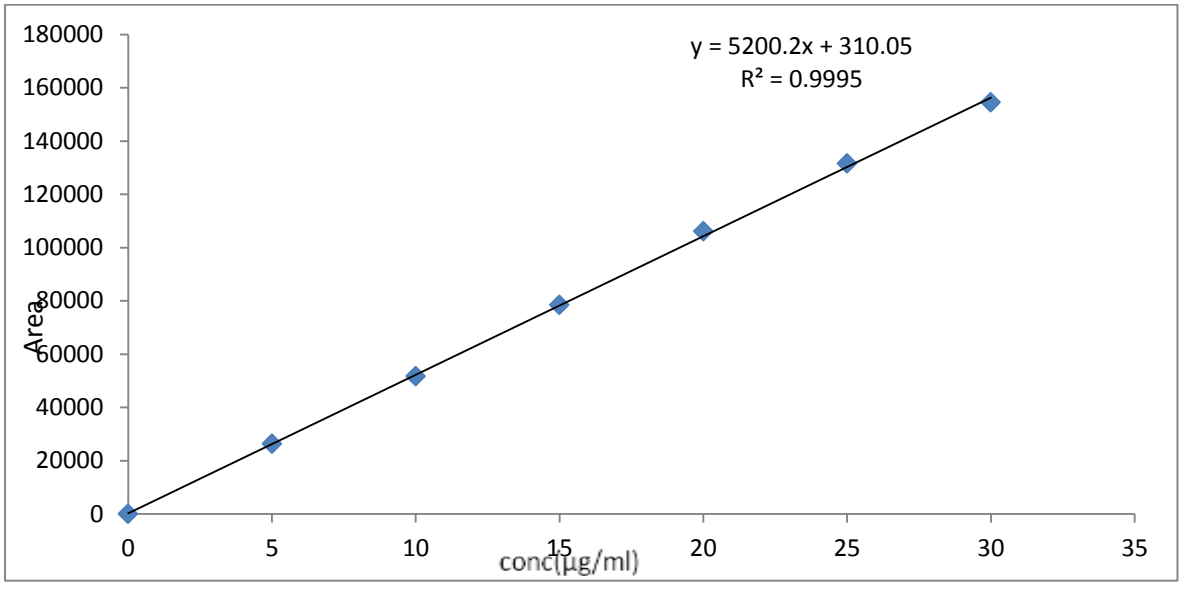

Fig. 4: Calibration curve of ivabradine

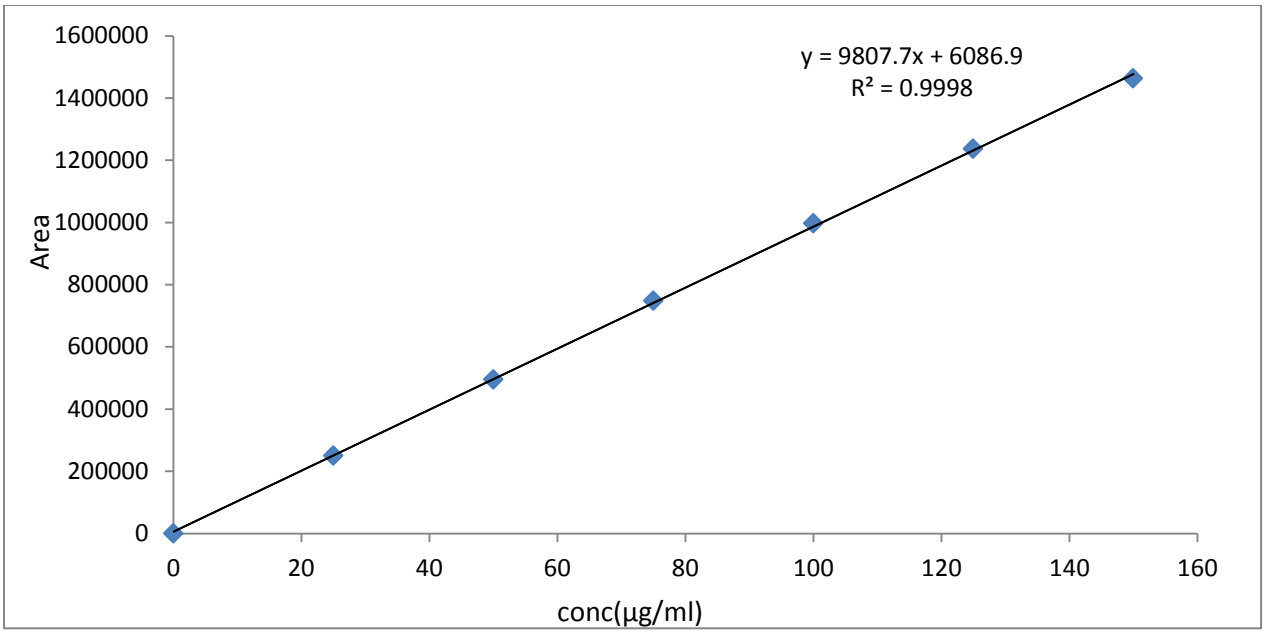

Fig. 5: Calibration curve of metoprolol

Table 2: Results for Linearity

Parameter	IVABRADINE	METOPROLOL
Y intercept	5200	9807
Intercept(c)	310.0	6086
Correlation coefficient (r^2)	0.999	0.999
Regression Equation	5200x+310.0	9807x + 6086
Linearity range	5-30µg/ml	25-150 µg/ml
LOD	0.11 µg/ml	0.39 µg/ml
LOQ	0.33 µg/ml	1.19 µg/ml

Accuracy

The Percentage accuracy was a relative standard deviation for accuracy at each level is well within the limit. Over all the percentage recovery of the relative standard deviation was found to be 98.55% - 101.02 % for all the levels was within the limit.

Table 3: Results for accuracy

IVABRADINE				METOPROLOL		
Recovery level	**Amount added (µg/ml)**	**Amount found**	**%Recovery (µg/ml)**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**% Recovery**
50%	10	9.98	99.83	50	50.51	101.02
100%	20	20.17	100.85	100	99.50	99.50
150%	30	30.22	100.73	150	147.83	98.55
Mean recovery			100.37%	Mean recovery		99.54%

Precision

Percentage relative standard deviation of six results was within the limit. Results shown good degree of precision was found to be 0.6% and 0.3%.

Limit of Detection

Limit of detection of target assay concentration of Ivabradine and Metoprolol by using formula method 0.11µg/ml and 0.33µg/ml within the limits.

Limit of Quantification

Limit of quantification of the target assay concentration of Ivabradine and Metoprolol by using formula method 0.39µg/ml and 1.19µg/ml were within the limits.

Robustness

In the above conditions, the parameters like % RSD of peak area, tailing factor and theoretical plates showed were within the limit.

Table 4: Robustness Conditions

S.No	Condition	Plus	Minus
1	Flow rate	0.9ml/min	0.7ml/min
2	Mobile phase	65B:35A	55B:45A
3	Temperature	35°C	25°C

Forced Degradation Study

Degradation studies demonstrated the specificity of the developed method in the presence of degradation products. Degradation was carried out in combination of two drugs and purity of drug peaks was confirmed by purity angles. Their combination drug products were exposed to acid, alkali, oxidative and thermal stress conditions. Then found to be no degradable substances presence and proved that the proposed method was stable towards acid, alkali, peroxide and thermal conditions within the limits.

Table 7: Results for stability studies of Ivabradine and Metoprolol

Parameters	Peak Area		% of degradation	
	Ivabradine	Metoprolol	Ivabradine	Metoprolol
Acid degradation	100277	908226	4.59	7.88
Alkaline degradation	100942	907647	3.96	7.94
Peroxide degradation	101633	920362	3.30	6.65
Thermal degradation	101941	959579	3.01	2.67
Photo Degradation	103306	969244	1.71	1.69

CONCLUSION

The RP-HPLC method developed and validated allows a simple and rapid quantitative determination of Ivabradine and Metoprolol in pharmaceutical dosage form. All the validation parameters were found to be within the limits according to ICH guidelines.

The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the short retention times allows the analyst to analyze no. of samples in a short period and method was found to be simple, accurate, precise, rugged, robust and stable under forced degradation stress conditions. So the established method can be successfully applied for the routine analysis of the marketed formulations.

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Conflict of interest: Nil

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