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The RP-UPLC method for simultaneous quantification of ivabradine and metoprolol

GV Subrahmanyam, JVLN SeshagiriRao, AKM Pawar

College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh- 530003
***Corresponding Author:GV Subrahmanyam**

ABSTRACT

In order to develop a newer or improved analytical method, the analyst has to set some goals. The method should be precise to the drug under study. It is necessary to determine the analyte at trace levels accurately. The UPLC techniques have now become extremely reliable and indispensable. Ivabradine, lowers the pacemaker firing rate, consequently lowering heart rate and reducing myocardial oxygen demand. Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. Run time was selected to be 3 min because the analysis gave peaks around 1.197 and 1.628 ±0.02 min of ivabradine and metoprolol. The analytical method was found to be linear over the range 1.25-7.5 µg/mL for ivabradine and 6.25-37.5 µg/mL for metoprolol of the target concentration.

Keywords:RP-UPLC, Ivabradine, Metoprolol, Precision, Accuracy

INTRODUCTION

Ultra Performance Liquid Chromatography spectroscopic detection is a powerful hyphenated technique for the analysis of drugs. Its sensitivity, accuracy and short analysis time make it ideal for determination of many drugs in dosage forms. Further, with the development of more sophisticated instrumentation, efficient column materials, sensitive detectors and moderate pricing, the UPLC techniques have now become extremely reliable and indispensable. In view of these advantages, the author has chosen to develop UPLC methods in this investigation for determination of some of selected drugs [1].

Ivabradine is a novel heart-rate lowering medicine for the symptomatic management of stable angina pectoris and chronic heart failure. Ivabradine (Corlanor®) was approved by the FDA in April 2015 for the treatment of chronic heart failure in patients who either is not on beta-blockers due to contraindications or is already receiving maximum beta-blocker dose. Recently, a new indication was added to treat symptomatic heart failure from dilated cardiomyopathy in patients of six months or more in age [2].

Ivabradine binds by entering and attaching to a site on the channel pore from the intracellular side and disrupts IF ion current flow, which prolongs diastolic depolarization and lowering the heart rate. The "IF" currents are located in the sino-atrial

node and are the home of all cardiac pacemaker activity.Ivabradine therefore, lowers the pacemaker firing rate, consequently lowering heart rate and reducing myocardial oxygen demand. This allows for an improved oxygen supply and therefore, mitigation of ischemia, allowing for a higher exercise capacity and reduction in angina episodes [3].

Metoprolol is a selective beta-1 blocker commonly employed as the succinate or tartrate derivative formulationdesigned for immediate release or extended release [4]. Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without showing activity on membrane stabilization [5].

MATERIALS AND METHODS

The reference standard samples of Ivabradine and Metoprolol were obtained from Matrix Ltd. Acetonitrile and Methanol used was of HPLC grade, while Sodium hydroxide, hydrogen peroxide was of GR grade (Merck Ltd. Mumbai, India). Milli-Q water was used throughout the analysis.The chromatograph consisted of a Waters Acquity H-Class UPLC (Model 2695) system equipped with a Hibar C18 (100 X 2.1mm; 2m) column, LC-20AD pumps and an SPD-20A photo diode array (PDA) detector. Samples were injected into the system through a Rheodyne 7725 injector valve via a 2µL loop. The output signal was monitored and integrated by Empower-2 software. Solubility of the compound was enhanced by sonication on a PCI Analytics PCI81 ultrasonicator. Weighing in the experiments was done on a Sartorius balance (model CPA225D). PVDF membrane filters used for filtration were purchased from Merck Millipore.

Preparation of the standard solution of Ivabradine and Metoprolol

5mg of Ivabradine and 25mg of Metoprolol were accurately weighed and transferred into a 100 mL clean dry volumetric flask. 50 mL of the diluent was added to it and sonicated for 5 min [6]. The final volume was made up with the diluent. This solution contains 50µg/mL of Ivabradine and 250µg/mL of Metoprolol.

Optimization of chromatographic conditions and method development

Under the below mentioned Table 1, the optimized conditions, the retention times obtained for Ivabradine and Metoprolol were 1.197 and 1.628 min respectively.

Table 1: Optimized chromatographic conditions of the proposed method

S. No.	Parameter	Value
1	Stationary phase	Hibar C18 (100 x 2.1mm; 2µ)
2	Mobile phase	Water: Methanol (70:30%)
3	Flow rate	0.5 mL/min
4	Column temperature	30ºC
5	Volume of injection	2µL
6	Detection wavelength (λ_{max})	260nm
7	Run time (min)	3min

System suitability

System suitability was assessed by analyzing the mixed standard drug solution (5µg/mL of Ivabradine and 25 µg/mL of Metoprolol) and then calculating the chromatographic parameters such as resolution, theoretical plates, and tailing factor [7].

Specificity

Specificity is the extent to which the procedure applies to the analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in

formulation did not interfere with the drug peaks and thus the method is specific [6].

Linearity

To establish the linearity, a stock solution containing 125µg/mL Ivabradine and 625µg/mL Metoprolol were prepared in the diluent to yield solutions in the concentration range of 1.25-7.50 µg/mL of Ivabradine and 6.25-37.5 µg/mL of Metoprolol and the solutions were analyzed in triplicateby injecting 2µL into the UPLC system [8].

Accuracy

To determine the accuracy of the proposed method, different amounts of Ivabradine and Metoprolol within linearity limits were taken and analyzed by the proposed method. Accuracy for Ivabradine and Metoprolol was conducted by spiking the drug to the pre-analyzed drug solutions at three different levels of the test concentration (i.e. 50%, 100%, and 150%) and three times at each level). The mean % Recovery and % RSD values were calculated [9].

Precision

To ascertain the effectiveness of method system suitability tests were carried out on freshly prepared solution containing 5µg/mL of Ivabradine and 25µg/mL Metoprolol. 2 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the peak response of sample were calculated [10].

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

LOD and LOQ values were calculated from the average standard deviation and slope from the calibration curve as per ICH guideline.

Robustness

Robustness study was done by applying small deliberate changes in the chromatographic conditions and studying the system suitability parameters of both the drugs. The conditions selected for testing were the flow rate, column oven temperature and composition of the mobile phase. The study was conducted on a mixed standard solution containing 5µg/ml of Ivabradine and 25µg/ml of Metoprolol [11].

RESULTS AND DISCUSSION

System suitability: It was represented in Table 2.

Table 2: System suitability values for the present method

Parameter	Ivabradine	Metoprolol
1. Retention time (min)	1.197	1.628
2. Peak area	129055	1248777
3. Resolution	-	3.7
4. Theoretical plates	3037	2871
5. Tailing Factor	1.19	1.20

Specificity

The UPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention time ranges. Figure 1a andFigure1 b show the representative chromatograms for standard and the formulation. The figures show that the selected drugs were clearly separated. Thus the proposed UPLC method is selective.

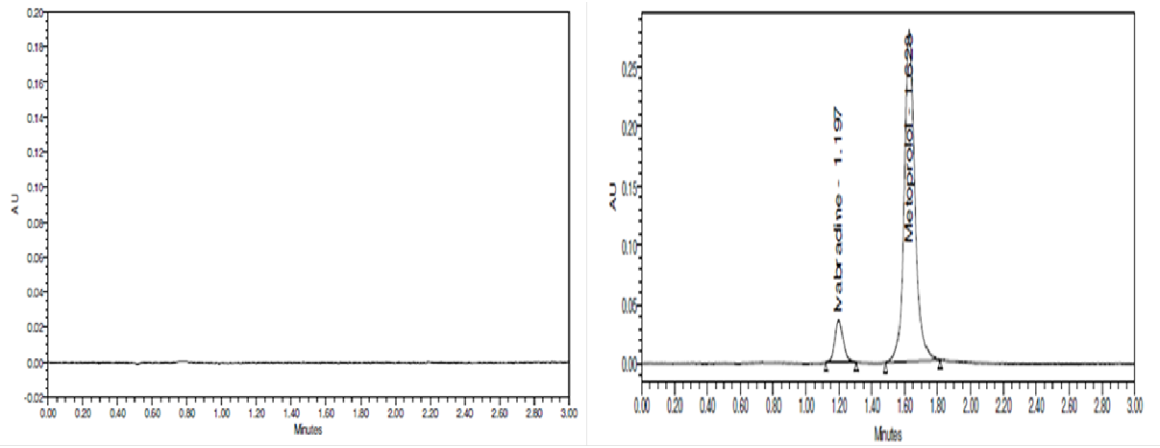

Figure 1: (a) A Typical Chromatogram of Placebo (b) A Typical Chromatogram of Ivabradine and Metoprolol in mixed standard solution

Linearity: Linearity data for Ivabradine and Metoprolol are given in the tables 3 and 4 respectively.

Table 3: Linearity of Ivabradine

Concentration of Ivabradine (µg/mL)	Peak Area	Mean Area	RSD
1.25	37049	36618	0.01
	36093		
	36711		
2.5	65554	66045	0.01
	66130		
	66452		
3.75	96205	96933	0.01
	96517		
	98078		
5	128342	129266	0.02
	132688		
	126768		
6.25	161301	161730	0.01
	163666		
	160224		
7.5	187904	189991	0.02
	194503		
	187565		

Table 4: Linearity data of Metoprolol

Concentration of Metoprolol (µg/mL)	Peak Area	Mean Area	RSD
6.25	365179	361744	0.01
	361967		
	358085		
12.5	645909	645573	0.00
	647449		
	643362		
18.75	961660	962324	0.00
	959856		
	965456		
25	1244208	1240731	0.00
	1236474		
	1241510		
31.25	1546533	1559153	0.01
	1560066		
	1570861		
37.5	1839853	1837172	0.00
	1833088		
	1838575		

Accuracy: The results are present in Table 5 and 6**.**The %Recovery value was found to be in between 98.0 % to 102.0 %.

Table 5: Recovery of Ivabradine

Concentration Level	Peak area difference	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Assay
50%	62891	2.5	2.511	100.44	99.5
	62008	2.5	2.476	99.04	
	62065	2.5	2.478	99.13	
100%	125351	5.0	4.985	99.71	99.79
	125980	5.0	5.010	100.21	
	125028	5.0	4.973	99.45	
150%	187234	7.5	7.437	99.16	99.80
	190389	7.5	7.562	100.83	
	187684	7.5	7.455	99.40	

Table 6: Recovery of Metoprolol

ConcentrationLevel	Peak area difference	Amount added (µg/mL)	Amount (µg/mL)	% Recovery	Mean % Assay
50%	615430	12.5	12.530	100.24	99.72
	610303	12.5	12.425	99.40	
	611001	12.5	12.439	99.51	
100%	1215959	25.0	24.892	99.57	99.62
	1215552	25.0	24.884	99.53	
	1218418	25.0	24.943	99.77	

150%	1824052	37.5	37.409	99.76	99.46
	1813733	37.5	37.197	99.19	
	1818042	37.5	37.286	99.43	

Precision

The inter-day precisions were determined by analyzing a mixed solution containing 50 µg/mL of Ivabradine and 250 µg/mL of Metoprolol. The intermediate precision was determined on two consecutive days different instrument. The results are depicted in the table 7.

Table 7: Inter-dayprecision data

S.No	Injection	Ivabradine		Metoprolol	
		Day-1	Day-2	Day-1	Day-2
1.	Injection-1	128857	128398	1245983	1235745
2.	Injection-2	128846	128016	1248772	1222607
3.	Injection-3	128989	129261	1251881	1235146
4.	Injection-4	129714	128175	1252272	1230375
5.	Injection-5	129006	128443	1243792	1238243
6.	Injection-6	128857	128014	1249960	1245535
Mean		128720		1241693	
SD		520.54		9313.99	
% RSD		0.40		0.75	

LOD and LOQ

The LOD and LOQ values for Ivabradine were found to be 0.12 and 0.37. The LOD and LOQ values for Metaprolol were found to be 0.05 and 0.15.

Robustness

The results remained unaffected by small variations in these conditions. The results were represented in tables 8 and 9.

Table 8: Robustness data of Ivabradine

Modified chromatographic conditions	Ivabradine			
	% assay	Theoretical Plates	Asymmetry	Retention time
Water: Methanol (60:40% v/v)	99.59	2914	1.1	1.191
Water: Methanol (80:20% v/v)	99.80	3002	1.2	1.427
0.4 mL/min	100.50	3014	1.1	1.429
0.6 mL/min	99.89	2917	1.1	1.029
28°C	99.25	2987	1.2	1.131
32°C	99.51	3011	1.1	1.100

Table 9: Robustness data of Metoprolol

Modified Chromatographic conditions	Metoprolol			
	% assay	Theoretical Plates	Asymmetry	Retention time
Water: Methanol (60:40% v/v)	101.41	2182	1.3	1.580
Water: Methanol (80:20% v/v)	99.20	2658	1.1	2.050

0.4 mL/min	99.58	2574	1.2	1.945
0.6 mL/min	98.9	2248	1.0	1.402
28°C	100.23	2687	1.2	1.847
32°C	99.79	2524	1.1	1.452

CONCLUSION

The present analytical method was developed by studying different parameters. The column used for the study was Hibar C18 (100 x 2.1mm; 2 m)because it gave good peak shapes. Ideal λ max for both the drugs was found to be 260 nm as the peak purity was good. Injection volume was selected to be 2µL which gave a good peak area. The flow rate was fixed at 0.5 mL/min for giving satisfactory retention times. A mixture of water andacetonitrile (50:50 v/v) was found to be ideal for the proposed study as it resulted in good resolution of the two drugs. Run time was selected to be 3 min because the analysis gave peaks around 1.197 and 1.628 ±0.02 min of ivabradine and metoprolol respectively. The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 1.25-7.5 µg/mL for ivabradine and 6.25-37.5 µg/mL for metoprolol of the target concentration. The analytical method passed both the robustness and ruggedness tests. In both the cases, relative standard deviation was below 2.0.

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