IJBPAS, May, 2021, 10(5): 1701-1719

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IVABRADINE AND METOPROLOL IN PHARMACEUTICAL DOSAGE FORM AND IN BLOOD PLASMA

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Received 20th June 2020; Revised 22nd July 2020; Accepted 27th Aug. 2020; Available online 1st May 2021

https://doi.org/10.31032/IJBPAS/2021/10.5.5489

ABSTRACT

A simple reverse phase HPLC method was developed for the simultaneous estimation of Ivabradine and Metoprolo in bulk and tablet form. Chromatography was performed by isocratic reverse phase separation on a stainless steel column 4.6 x 150mm, symmetry column packed with octa decyl silane bonded to porous silica (C18) with particle size 5 micron with mobile phase containing Methanol and phosphate buffer in the ratio of 50:50. The flow rate was 0.8 ml/ min and effluent was monitored at 260 nm. The retention times were 2.24 min and 2.66 min Metoprolol and Ivabradine respectively. The standard curve was linear over a working range of 25–150 µg/ml for Metoprolol and 5-30 µg/ml for Ivabradine and gave an average correlation coefficient of 0.999, and 0.999 for Metoprolol and Ivabradine respectively. The limit of quantitation (LOQ) of this method was 0.56 µg/ml and 3.8 µg/ml for Metoprolol and Ivabradine respectively. The absolute recovery was 99.96% for Metoprolol and 99.86 for Ivabradin. Degradation products produced as a result of stress studies did not interfere with the detection of Metoprolol and Ivabradine, the assay can thus be considered stability-indicating.

Keywords: Metoprolol, Ivabradine, RP-HPLC, Phosphate Buffer, Methanol, Validation

INTRODUCTION

Metoprolol is a beta-blocker that affects the heart and circulation (blood flow through arteries and veins). Metoprolol is used to treat angina (chest pain) and hypertension (high blood pressure). Metoprolol is also used to lower your risk of death or needing to be hospitalized for heart failure **[3, 4]**.

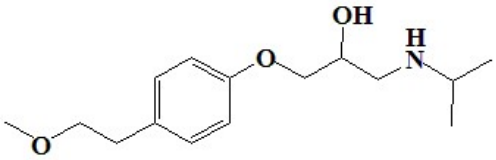

Figure 1: Metoprolol

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 presenting activity towards membrane stabilization nor intrinsic sympathomimetics **[1]**.

Ivabradine is a novel heart rate lowering medicine for the symptomatic management of stable angina pectoralis and symptomatic chronic heart failure. Ivabradine was approved by the FDA in for the treatment of chronic heart failure in patients with an ejection fraction of ≤35%, in sinus rhythm with resting heart rate ≥70 beats per minute, who are not on beta-blockers due to contraindications or already receiving maximum beta-blocker dose **[1, 2]**.

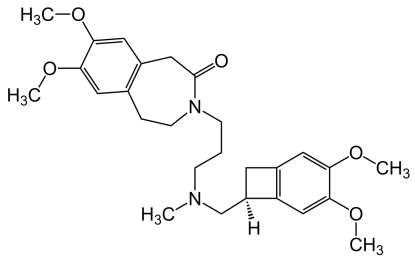

Figure 2: Ivabradine

Ivabradine lowers heart rate by selectively inhibiting If channels ("funny channels") in the heart in a concentration-dependent manner without affecting any other cardiac ionic channels (including calcium or potassium). 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, lowering heart rate. The currents are located in the sinoatrial 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.

MATERIALS AND METHODS

Drugs

Pure pharmaceutical sample of Ivabradine (IVR) and Metoprolol (MET) was obtained

from Yucca Pharma. Commercial tablet of Ivabradine (IVR) (5mg), Metoprolol (25mg) Ivamet XL 5 mg/25mg were procured from the local drug market **[6-9]**.

Chemicals

Potassium dihydrogen phosphate (AR Grade), 85% Orthophosphoric acid (AR Grade), Methanol (HPLC Grade), Orthophosphoric acid (AR Grade), Triethyl-Amine (AR Grade), Sodium Hydroxide (AR Grade) were purchased from Sd fine-Chem limited **[3]**.

Instrument

Liquid chromatographic system from Waters alliance 2695 with Waters UV detector equipped with Empower software was used.

Preparation of mobile phase:

Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Mobile phase:

Accurately measured 500 ml (50%) of above buffer and 500 ml of Methanol HPLC (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration **[5]**.

Diluent Preparation:

The Mobile phase was used as the diluents**.**

Preparation of Standard stock solutions: Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 50ml 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 and labeled as Standard stock solution 1and 2. (50µg/ml of Ivabradine and 250µg/ml of Metoprolol)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (5µg/ml Ivabradine of and 25µg/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 100 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 (50µg/ml of Ivabradine and 250µg/ml of Metoprolol).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (5µg/ml of Ivabradine and 25µg/ml of Metoprolol).

Validation Parameter

Linearity:

Preparation of Standard stock solutions: Accurately weighed 2.5 mg of Ivabradine, 12.5mg of Metoprolol and transferred to individual 50 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 and labeled as Standard stock solution 1and 2. (50µg/ml of Ivabradine and 250µg/ml of Metoprolol)

25% Standard solution: 1 ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Ivabradine and 25 µg/ml of Metoprolol).

50% Standard solution: 2 ml each from two standard stock solutions was pipetted out and made up to 10ml. (10 µg/ml of Ivabradine and 50 µg/ml of Metoprolol).

75% Standard solution: 3 ml each from two standard stock solutions was pipetted out and made up to 10ml. (15 µg/ml of Ivabradine and 75 µg/ml of Metoprolol).

100% Standard solution: 4 ml each from two standard stock solutions was pipetted out and made up to 10ml. (20 µg/ml of Ivabradine and 100 µg/ml of Metoprolol).

125% Standard solution: 5 ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Ivabradine and 125 µg/ml of Metoprolol).

150% Standard solution: 6 ml each from two standard stock solutions was pipettede out and made up to 10ml. (30 µg/ml of Ivabradine and 150 µg/ml of Metoprolol).

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 2.5 mg of Ivabradine, 12.5 mg of Metoprolol and transferred to individual 50 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 and labeled as Standard stock solution 1and 2. (50µg/ml of Ivabradine and 250µg/ml of Metoprolol)

Preparation of 50% Spiked Solution: 1 ml of sample stock solution was taken into a 10ml volumetric flask, to that 0.5 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml

from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Ivabradine, Metoprolol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ivabradine, Metoprolol, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Apparatus and Chromatographic conditions:

Quantitative HPLC was performed on Waters HPLC system with UV detector. empower software is used along with a stainless steel column 4.6 x 150mm, packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. To develop a suitable and robust HPLC method for the determination of IVR and MET, different mobile phases containing buffer and Methanol were used in different compositions like (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5,0.75,1.0, 1.2, 1.5, ml/min). The mobile phase containing buffer and methanol with a flow rate of 0.8 ml/ min gave peaks of good resolution and were eluted at retention times around 2.23 min, 2.65 min with symmetric peak shape. The detection is performed at the wavelength 260 nm **[6-9]**.

Running the standard solution of Metoprolol and Ivabradine

1 ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Ivabradine and 25µg/ml of Metoprolol). The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in **Figure 3**.

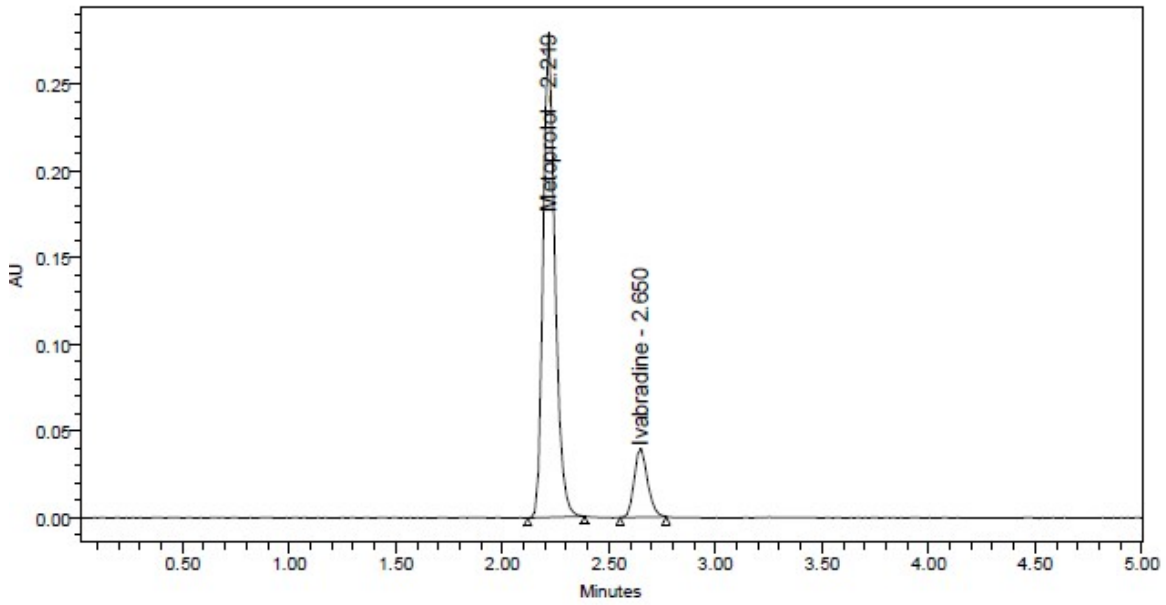

Figure 3: Chromatogram of Metoprolol (Rt-2.21 min) and Ivabradine (Rt-2.66min)

S. No.	Name	Retention time(min)	Area (µV sec)	USP tailing	USP plate count
1	Metoprolol	2.21	1089296	1.26	7567
2	Ivabradine	2.66	170797	1.16	9151

RESULTS AND DISCUSSION

Method development and optimization: The main target of the chromatographic method is to get the separation of closely eluting drugs Metoprolol and Ivabradine, The drugs were co-eluted by using different stationary phases like C18, C8 with varying lengths and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-7) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. pH of the buffer has played a significant role in achieving the separation between drugs.

The chromatographic separation was achieved on a stainless steel column (4.6 x 250mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron, by using solutions phosphate buffer and methanol in the ratio of (50:50), pH adjusted to 4 using ortho phosphoric acid. The flow rate of the mobile phase was maintained at 0.8 ml/min. At 25^0 C of column temperature, the peak shape of MET and IVR was found symmetrical with mobile phase 50:50 ratio. In the optimized conditions MET and IVR were well separated with a good resolution and the

typical retention times of MET and IVR were about 2.2 min and 2.6 min, respectively. The system suitability results are given in **Table 1** and the developed LC method was validated **[10-14]**.

Results of method validation

Linearity: Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 1- 37.5 µg/ml for Metoprolol and 1µg/ml to 7.5 µg/ml for Ivabradine and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte which is given in **Table 2 and 3, Figure 4, 5**.

Precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions **(Table 4, 5, 6)**.

System Precision (Table 6)

Recovery and accuracy: The percentage recovery of MET and IVR in bulk drugs samples was ranged from 99.96 - 99.86% which indicates that the method was accurate which is given in **Table 7**.

Accuracy results:

The accuracy of the method was determined by preparing solutions of different concentrations of MET and IVR that is 50%, 100% and 150% in which the amount of marketed formulation (MET and IVR 20 µg and 100 µg respectively) was kept constant and the amount of pure drug was varied that is 10 µg, 20 µg and 30 µg for IVR and 50 µg, 100 µg and 150 µg for MET i.e. 50%, 100% and 150% respectively. The solutions were prepared in triplicates and the accuracy. similarly was indicated by % recovery in **Table 7 and 8**.

LOD and LOQ: Detection limit and Quantitation limit of described method were observed as given in (Table 9).

ROBUSTNESS:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength (235 and 239 nm) and mobile phase flow rate by 0.1 ml/min (0.9 and 1.0ml/min) had no significant effect on the retention time and

chromatographic response of the 20 µg/ml and 100 µg/ml for ivabradine and Metoprolol solution respectively, indicating that the method was robust.

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & methanol content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (**Table 10, 11**; % RSD < 2%) the developed RP-HPLC method for the analysis of Amlodipine.

STABILITY STUDY:

I. Acid Hydrolysis:

Accurately weighed 25 mg of Ivabradine, 25mg 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 and labeled as standard solution 1 and 2. (1 mg/ml of Ivabradine and Metoprolol) **(Figure 6)**.

The mixture of Ivabradine and Metoprolol (1 mg/ml) was treated with 2 ml 0.1N HCl and heated at 60°C for 6 hr. The mixture was cooled at room temperature**.** The resultant solution was neutralized and diluted to obtain 20ppm and 100ppm of Ivabradine and Metoprolol solution respectively. 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample **[15-17]**.

II. Basic Hydrolysis

The mixture of Ivabradine and Metoprolol (1 mg/ml) was treated with 2 ml 0.1 N sodium and heated at 60°C for 6 hr. The mixture was cooled at room temperature**.** The resultant solution was neutralized and diluted to obtain 20ppm and 100ppm of Ivabradine and Metoprolol solution respectively. From the resultanty solution 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample **(Figure 7)**.

III. Dry Heat Degradation

The mixture of Ivabradine and Metoprolol (1 mg/ml) was placed in oven at 80^{0}C for 24 hour to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 20ppm & 100ppm of Ivabradine, & Metoprolol solution respectively. From the resultant solution 10µl were injected into the system, the chromatograms obtained were recorded to assess the stability of the sample **(Figure 8)**.

IV. Photolytic Degradation:

To study the photochemical stability the mixture of Ivabradine and Metoprolol (1

mg/ml) drug was exposed to UV Light (254 nm and 366 nm) by keeping the drug in UV Chamber for 24 hours. For HPLC study, the resultant solution was diluted to obtain (20ppm & 400ppm) solutions and 10 µl were injected into the system. The chromatograms were recorded to assess the stability of sample **(Figure 9)**.

V. Oxidation With (3%) H_2O_2:

The mixture of Ivabradine and Metoprolol (1 mg/ml) was added with 2 ml of 3% hydrogen peroxide (H_2O_2) and the mixture was refluxed at 60^0C for 6 hours. For HPLC study, the resultant solution was diluted to obtain (20ppm, & 100ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample **(Figure 10)**.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to (20ppm & 100ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample **(Figure 11)**.

Results of forced degradation studies:

The results of the stress studies indicated the **specificity** of the method that has been developed. Ivabradine and Metoprolol were stable in photolytic, thermal and basic stress conditions. The result of forced degradation studies are given in the following **Table 12**.

Results of Plasma studies:

The mixture of drug sample containing Ivabradine 0.2 mg and Metoprolol 1 mg was introduced orally in the 3 rats. Animal was allowed to access to food 4 h after dose administration. 1 ml of blood sample was withdrawn from lateral tail vein with help of heparinise catheter and syringe at time intervals of 0 (pre-dose), 4, 6, 8, 12,and 24 hr post administration. The plasma was separated by centrifugation at 4000 rpm for 15 min and was stored at -20°C till analysis.

Plasma samples (1 mL) in 13 × 100-mm glass test tubes. was treated with of 10M sodium hydroxide solution. The sample obtained from Liquid– liquid extraction is injected into the chromatographic system for quantitation **(Figure 12-15)**.

Table 1: System suitability parameters

Instrument used	**: Waters HPLC with auto sampler and UV detector**
Temperature	**Ambient**
Column	**Inertsil C18 (4.6 x 150mm, 5µm)**
Buffer :	**Accurately weighed 6.8 grams of KH2PO4 dissolved 1000ml. volume was adjusted to pH 4.0 with Orthophosphoric acid**
pH	**4**
Mobile phase	**50% buffer and 50% Methanol**
Flow rate	**0.8 ml per min**
Wavelength	**260 nm**
Injection volume	**20 µl**
Run time	**10 min**

Table 2: Linearity results: (for Metoprolol)

Concentration of MET in ppm	Peak area
0	**0**
25	**284011**
50	**565789**
75	**803165**
100	**1078798**
125	**1308468**
150	**1592569**

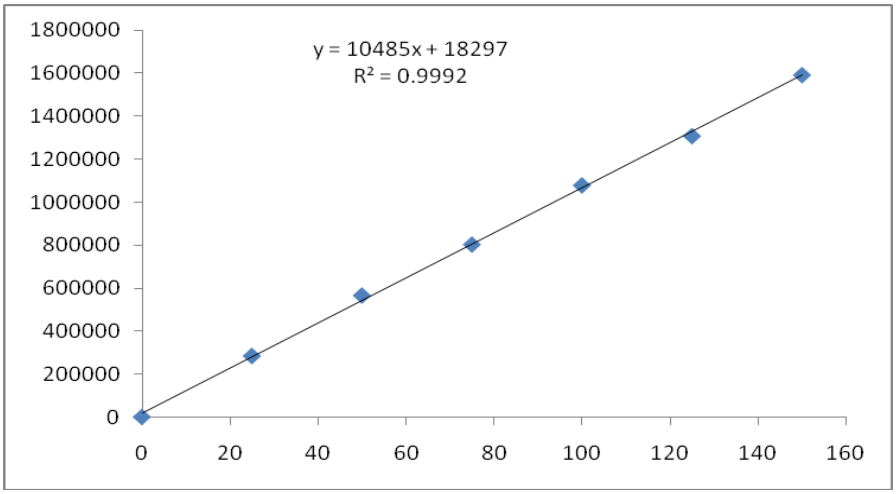

Figure 4: Calibration curve of Metoprolol

Table 3: Linearity results: (for Ivabradine)

Concentration of IVR in ppm	Peak area of Olmesartan
0	**0**
5	**42951**
10	**86790**
15	**129434**
20	**172080**
25	**218488**
30	**255893**

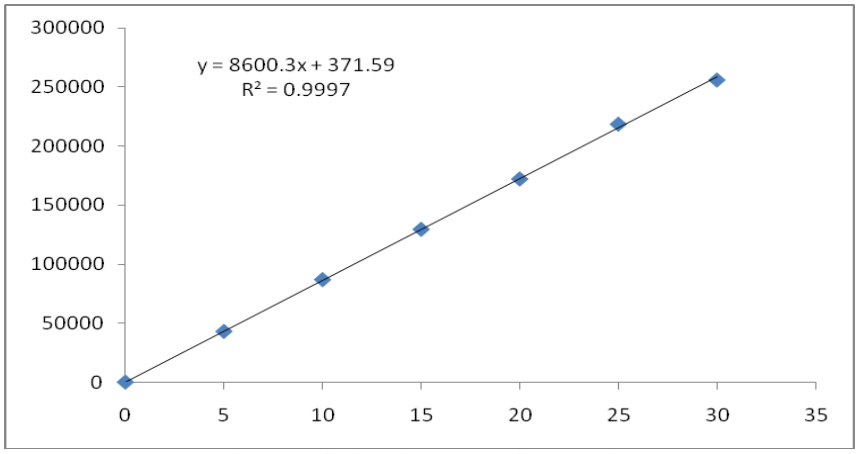

Figure 5: Calibration curve of Ivabradine

Table 4: Results of Intermediate precession for Metoprolol

S. No.	Peak name	Rt	Area (µV*sec)	USP Plate Count	USP Tailing
1	Metoprolol	2.23	990531	7818	1.23
2	Metoprolol	2.23	995548	7768	1.25
3	Metoprolol	2.23	989696	7490	1.25
4	Metoprolol	2.24	992954	7757	1.23
5	Metoprolol	2.23	990770	7758	1.25
6	Metoprolol		994301	7470	1.23
	Mean		992300		
	Std. Dev		2333.8		
	% RSD		0.2		

Table 5: Results of Intermediate precession for Ivabradine

S. No.	Peak Name	Rt	Area (µV)	USP Tailing	USP Plate Count
1	Ivabradine	2.65	158333	1.17	8425
2	Ivabradine	2.66	158364	1.16	8940
3	Ivabradine	2.65	159749	1.16	9081
4	Ivabradine	2.66	160368	1.15	8732
5	Ivabradine	2.66	159388	1.14	8854
6	Ivabradine	2.65	157989	1.16	8770
	Mean		159032		
	Std. Dev.		943.2		
	% RSD		0.6		

Table 6: System precision table of Ivabradine and Metoprolol

S. No	Area of Ivabradine	Area of Metoprolol
1.	172986	1065156
2.	172721	1059477
3.	172916	1063748
4.	173381	1060344
5.	173028	1060037
6.	174028	1052631
Mean	173177	1060232
S.D	469.2	4359.0
%RSD	0.3	0.4

Table 7: Accuracy studies for Ivabradine

% Concentration	Area	Amount added (µg)	Amount Found (µg)	% recovery	Mean Recovery
50%	262339	10	10.07	100.73	99.86%
	261113	10	9.93	99.30	
	260937	10	9.91	99.10	
100%	348205	20	20.06	100.28	
	346672	20	19.88	99.39	
	346504	20	19.86	99.30	
150%	435032	30	30.15	100.51	
	432723	30	29.88	99.62	
	435074	30	30.16	100.53	

Table 8: Accuracy results for Metoprolol

% Concentration (at specification Level)	Area	Amount added (mg)	Amount Found (mg)	% recovery	Mean Recovery
50%	1595668	50	50.44	100.88	99.96%
	1590916	50	49.99	99.98	
	1591791	50	50.07	100.14	
100%	2101313	100	98.67	98.67	
	2109097	100	99.41	99.41	
	2109840	100	99.48	99.48	
150%	2616195	150	147.77	98.52	
	2638896	150	149.94	99.96	
	2630008	150	149.09	98.52	
				99.96	
				99.39	

Table 9: LOD and LOQ

Molecule	LOD	LOQ
Ivabradine	1.25	3.80
Metoprolol	0.19	0.56

Table 10: Robustness data for Metoprolol

S. No.	Condition	%RSD of Metoprolol
1	Flow rate (-) 0.9ml/min	0.9
2	Flow rate (+) 1.1ml/min	1.0
3	Mobile phase (-) 55B:45A	1.1
4	Mobile phase (+) 45B:55A	1.2
5	Temperature (-) 25°C	0.8
6	Temperature (+) 35°C	0.7

Table 11: Results of robustness for and Ivabradine

S. No.	Condition	% RSD of Ivabradine
1	Flow rate (-) 0.9ml/min	0.8
2	Flow rate (+) 1.1ml/min	0.7
3	Mobile phase (-) 55B:45A	0.7
4	Mobile phase (+) 45B:55A	0.7
5	Temperature (-) 25°C	0.8
6	Temperature (+) 35°C	1.0

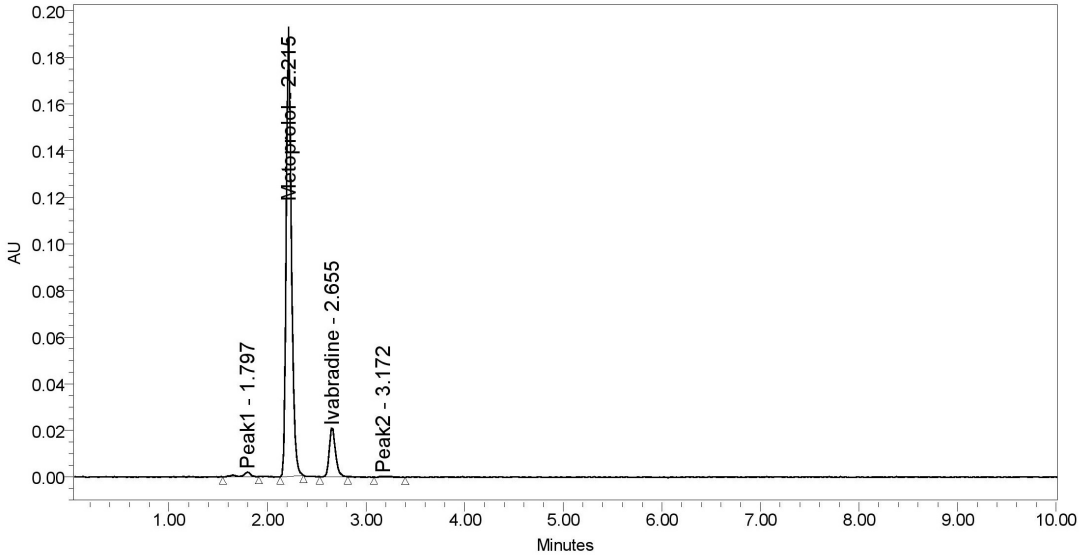

Figure 6: Chromatogram showing degradation for Metoprolol and Ivabradine in 0.1 N HCl

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Peak 1	1.79	11756	0.6	5165
2	Metoprolol	2.21	1024382	1.2	8193
3	Ivabradine	2.655	162874	1.2	8646
4	Peak 2	3.17	2937	2.3	14948

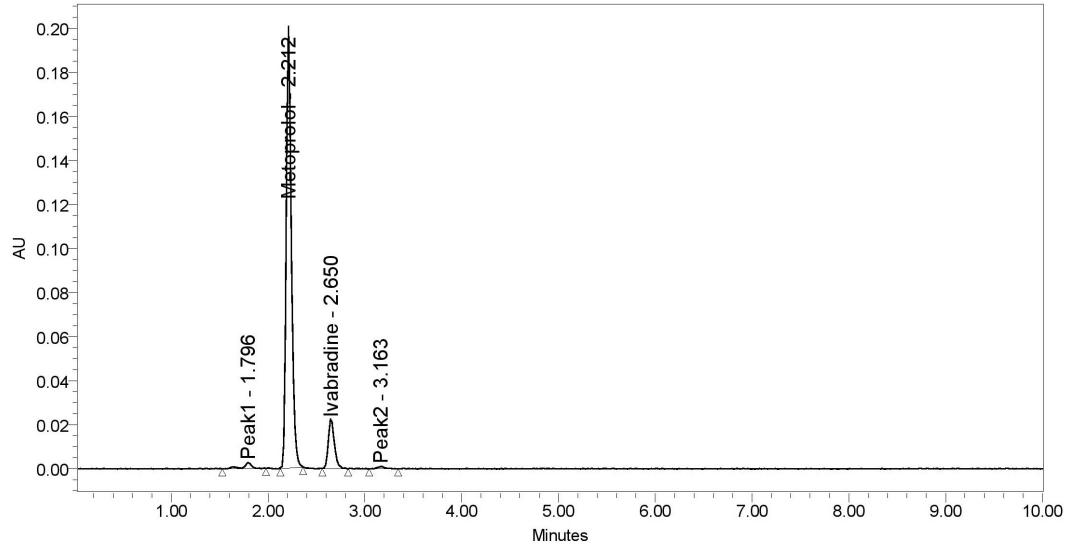

Figure 7: Chromatogram showing degradation related impurity in 0.1 N NaOH

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Peak 1	1.79	14618	0.7	4758
2	Metoprolol	2.21	1029339	1.2	8058
3	Ivabradine	2.65	163416	1.2	8387
4	Peak 2	3.16	4987	1.1	6992

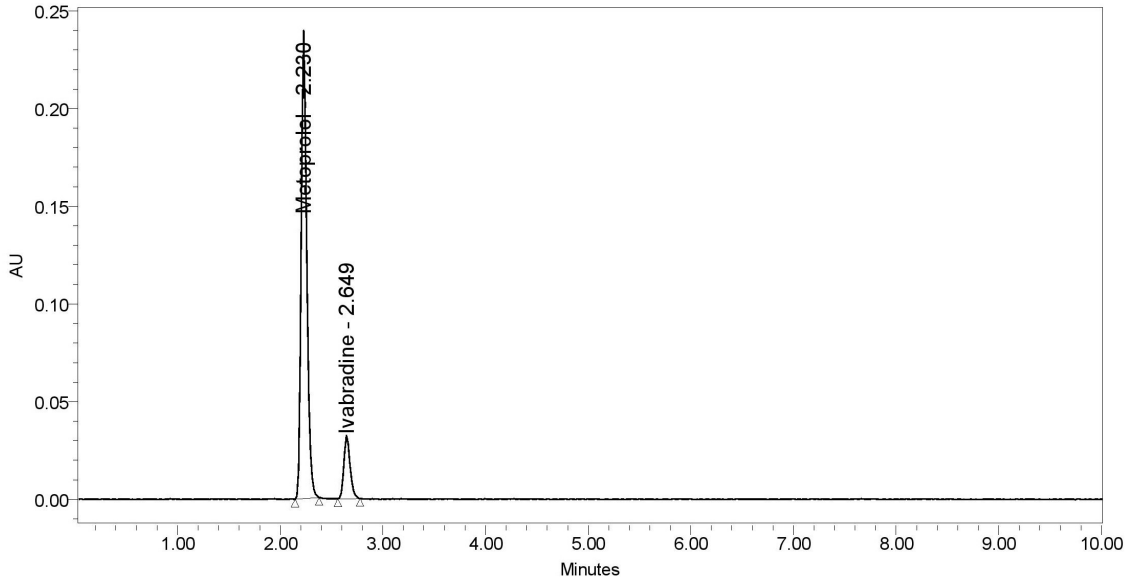

Figure 8: Chromatogram showing thermal degradation studies

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Metoprolol	2.23	1039367	1.2	7991
2	Ivabradine	2.64	168933	1.2	9083

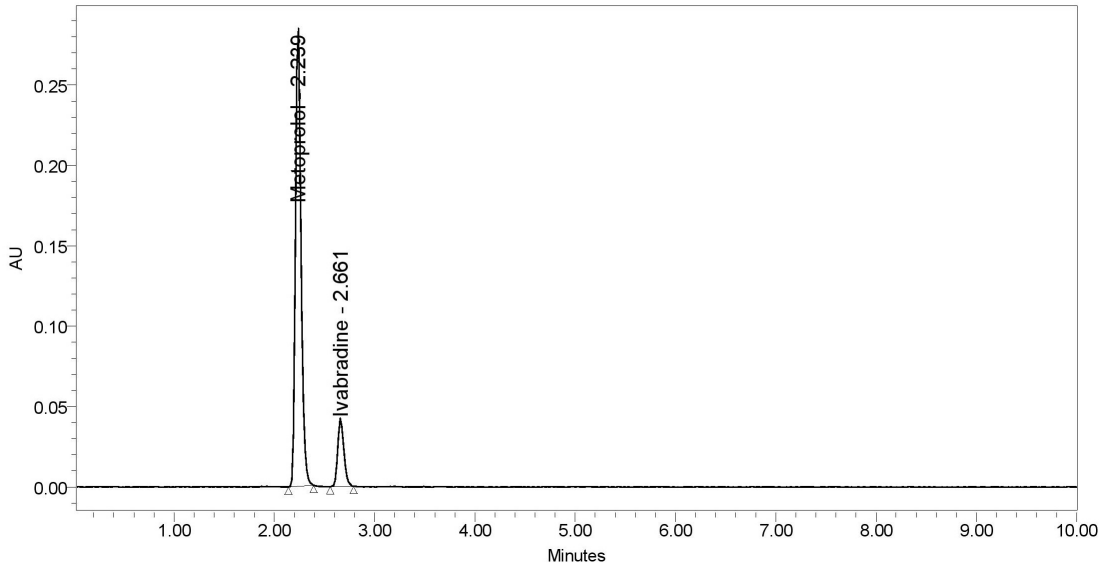

Figure 9: Chromatogram is showing photolytic degradation

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Metoprolol	2.23	1048258	1.2	7661
2	Ivabradine	2.66	170740	1.2	9287

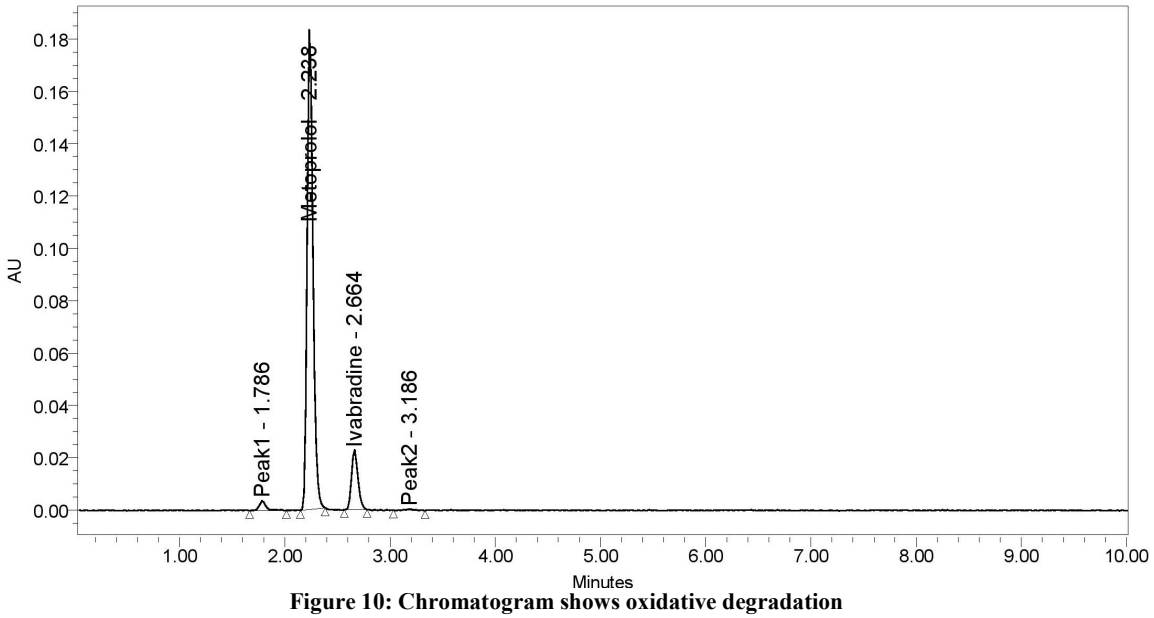

Figure 10: Chromatogram shows oxidative degradation

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Peak 1	1.78	14618	1.3	4195
2	Metoprolol	2.23	1029339	1.2	7632
3	Ivabradine	2.66	162691	1.2	9155
4	Peak 2	3.16	4987	1.6	2181

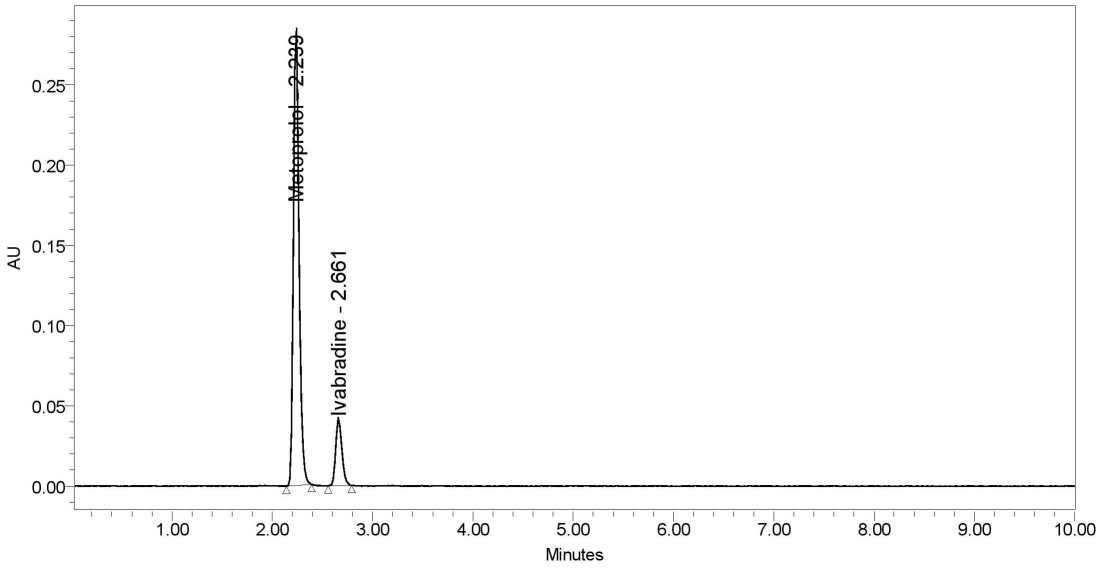

Figure 11: Chromatogram shows neutral degradation

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Metoprolol	2.23	1060581	1.2	7651
2	Ivabradine	2.66	172556	1.2	9187

Table 12: Results of forced degradation studies of Ivabradine and Metoprolol

Type of degradation	Ivabradine			Metoprolol		
	Area	%recovered	% degraded	Area	%recovered	% degraded
Acid (0.1 M HCl)	162874	93.86	6.14	1024382	96.23	3.77
Base (0.1 M NaOH)	163416	94.18	5.82	1029339	96.70	3.30
Peroxide (3 % H_2O_2)	162691	93.16	6.24	1033319	97.07	2.93
Thermal (80 ^{0}C)	168933	9735	2.65	1039367	97.64	2.36
Uv 254nm	170740	98.40	1.60	1048258	98.48	1.52
Water	172556	99.44	0.56	1060581	99.63	0.37

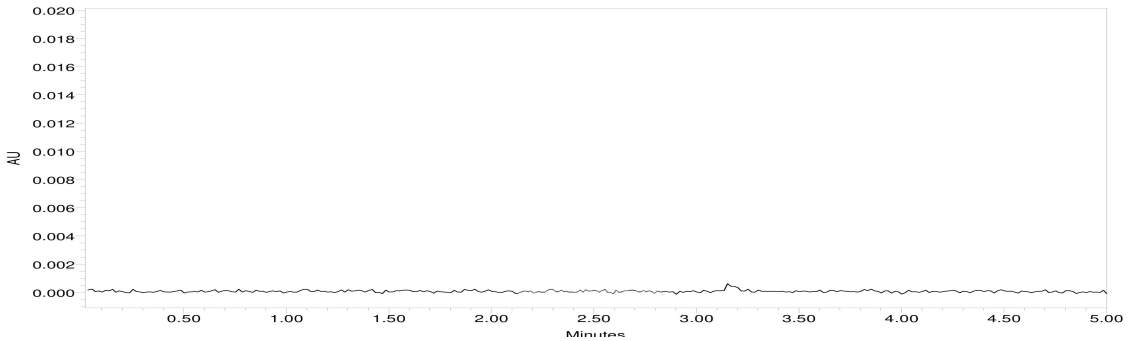

Figure 12: Chromatogram of blank plasma

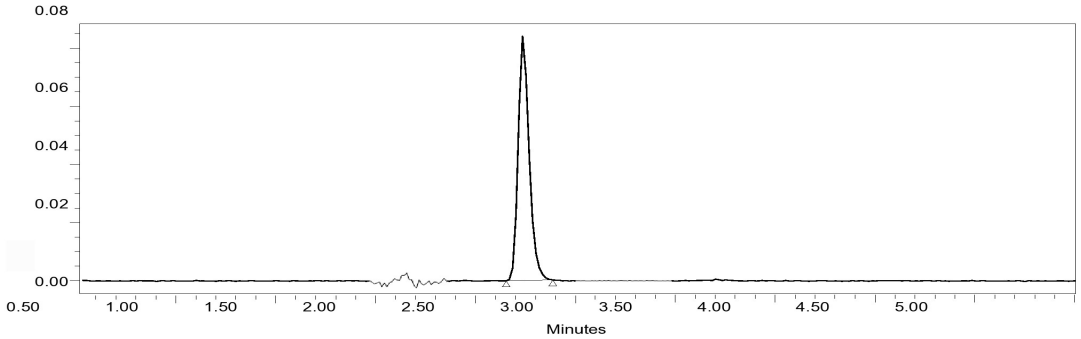

Figure 13: Chromatogram shows standard drug Metoprolol spiked with plasma

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Metoprolol	2.24	281436	1.2	7822

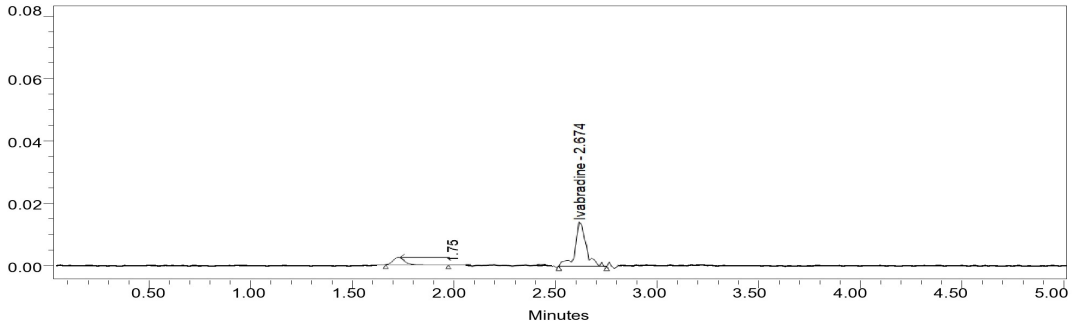

Figure 14: Chromatogram shows standard drug Ivabradine spiked with plasma

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Peak 1	1.75	11722	0.6	4985
2	Ivabradine	2.67	42925	1.12	9245

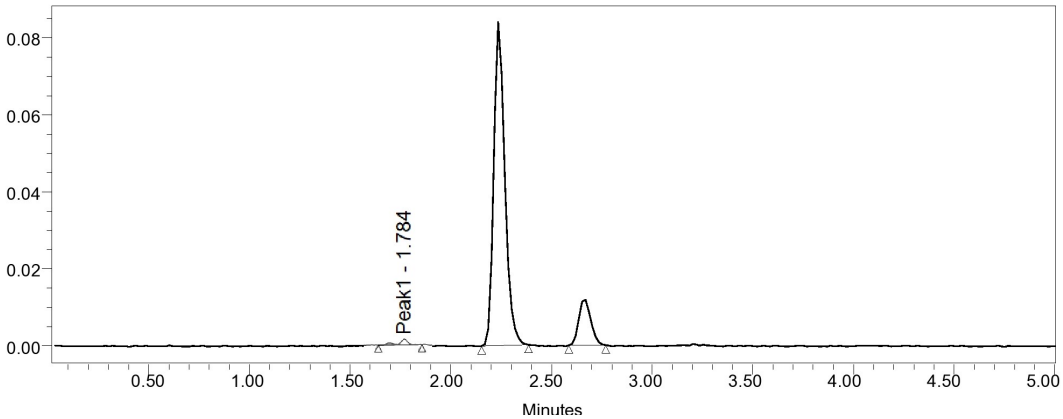

Figure 15: Chromatogram shows standard drug Ivabradine & Metoprolol spiked with plasma

S. No.	Peak Name	R_t	Area	USP Tailing	USP plate count
1	Peak 1	1.784	11756	0.6	4985
2	Metoprolol	2.24	281487	1.18	8142
3	Ivabradine	2.67	43006	1.10	8936

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Ivabradine and Metoprolol was done by RP-HPLC. The proposed method was found to be simple, precise, accurate and rapid for determination of Ivabradine and Metoprolol in pure and dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement within the limit. Hence, this method can be easily and conveniently adopted for routine analysis of Ivabradine and Metoprolol in pure form, dosage form and in plasma

ACKNOWLEDGEMENTS

I, Dhiraj Kumar, thankful to Dr. P. Suresh, Associate Director, School of Pharmacy, GNITC Campus, Ibrahimpattnam, Hyderabad, for providing necessary facilities to carry out the research work.

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