

**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR
ESTIMATION OF IVABRADINE AND METOPROLOL IN PURE AND
PHARMACEUTICAL DOSAGE FORM**

K. Sai Durga*, L. Ramachandra Reddy, D. Dhachinamoorthi

Department of Pharm. Analysis and Quality Assurance, QIS College of Pharmacy, Ongole-523272.

*Corresponding Author: K. Sai Durga

Department of Pharm. Analysis and Quality Assurance, QIS College of Pharmacy, Ongole-523272.

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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 Discovery C8 250mm x 4.6 mm, 5 μ m. Mobile phase containing KH₂PO₄: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0nm. Retention time of Ivabradine and Metoprolol were found to be 2.517min and 3.097min. %RSD of the Ivabradine and Metoprolol were found to be 0.6 and 0.3 respectively. %Recovery was obtained as 99.65% and 99.39 % for Ivabradine and Metoprolol respectively. LOD, LOQ values obtained from regression equations of Ivabradine and Metoprolol were 0.31, 0.95 and 0.68, 2.06 respectively. Regression equation of Ivabradine is $y = 17301x + 1713$ and $y = 17366x + 3213$ of Metoprolol. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEY WORDS: Ivabradine, Metoprolol, RP-HPLC.

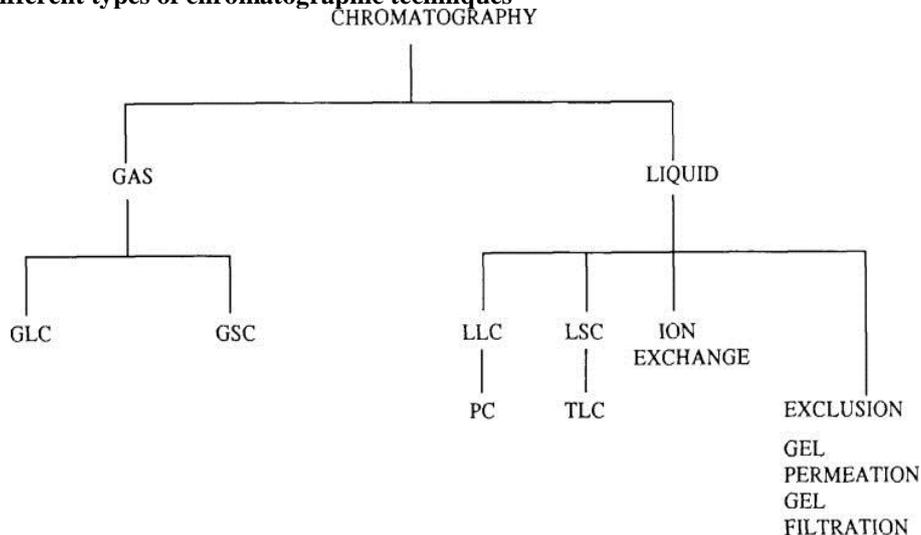
1. INTRODUCTION

Quality confirmation and control of pharmaceutical and compound plans is fundamental for guaranteeing the accessibility of protected and viable medication definitions to shoppers. Henceforth Analysis of unadulterated medication substances and their pharmaceutical measurements frames involves a crucial job in surveying the reasonableness to use in patients.

1.1 CHROMATOGRAPHY

Mixture of the components separated to individual based on differential partitioning between the mobile and stationary phases. Variations in analyte partition coefficient results in differential retention on the stationary phase and thus altering the separation. Different types of chromatographic techniques were summarized in

Table No. 1.1 Different types of chromatographic techniques



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

All LC methods HPLC method plays vital rule and it fastest growing one why because it gives accurate and stable results and also easy, less time and cost consuming.

Applications of HPLC in pharmaceutical research

- Separation
- Identification

- Quantification
- Isolation

1.4) HPLC theory

System suitability parameters

To determine the suitability and effectiveness of the optimized method measured by this test. In variables are used like retention time, USP plate count, Asymmetry factor and resolution.

Retention time (tR), capacity factor k' and relative retention time (RRT)

capacity factor k'	Retention time (tR),
It is a measure of the retention of a peak that is independent of column geometry or mobile phase flow rate	It is a measure of the time taken for a salute to pass through a chromatography column

DRUG PROFILE^[11-12]

Ivabradine

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 (I_f) in the sinoatrial hub in a portion subordinate design, bringing about a lower pulse and in this manner more blood to stream to the myocardium¹¹. 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 inotropic impacts

Structure

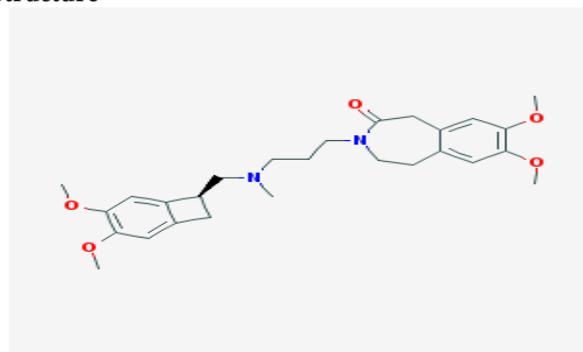


Fig. 1.1: Structure of Ivabradine.

Metoprolol

Metoprolol is a cardioselective β_1 -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 β_1 -adrenergic receptors with little activity against β_2 -adrenergic receptors of the lungs and vascular smooth muscle.^[12]

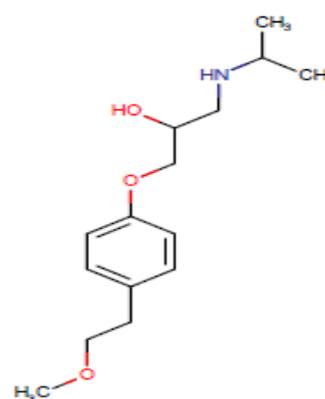


Fig. 2: Chemical structure of Metoprolol.

MATERIALS AND METHODS

Materials

- Ivabradine and Metoprolol pure drugs (API), Combination Ivabradine and Metoprolol tablets (IVA Met), Distilled water, Acetonitrile, Methanol. All the above chemicals and solvents are from Rankem.

Instruments

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Ivabradine and Metoprolol solutions.

Methods

OPTIMIZED METHOD

Buffer: 0.01N Potassium dihydrogen ortho phosphate (3.0pH)

Accurately weighed 1.36gm of Potassium dihydrogen 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.

LABEL CLAIM: 5mg/25mg Tablet

Brand name: IVAMET XL

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 and labeled as Standard stock solution 1 and 2. (200µg/ml of Ivabradine and 1000µg/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. (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 (100µg/ml of Ivabradine and 500µg/ml of Metoprolol).

Preparation of Sample working solutions (100% solution): 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).

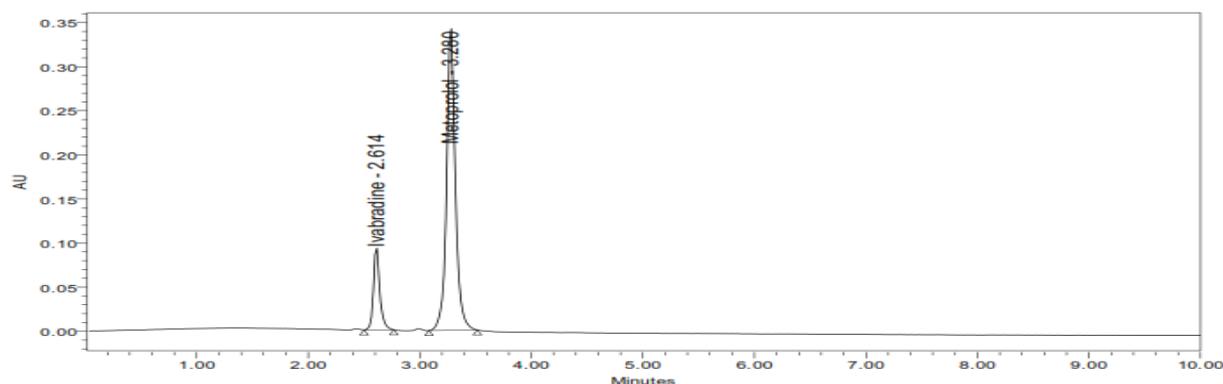
RESULTS AND DISCUSSION

Method development: Method development was done by changing various, mobile phase ratios, buffers and columns etc.

Optimized method

Optimized Chromatographic Conditions

Column : Discovery C8 250mm x 4.6 mm, 5µm.
 Mobile phase : KH₂PO₄: Acetonitrile (55:45)
 Flow rate : 1.0 ml/min
 Detector : PDA 260nm
 Temperature : 30⁰C
 Injection Volume : 10µL
 Run time : 10.0 min



Observation: Ivabradine and Metoprolol were eluted at 2.614 min and 3.280 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table: 6.1 System suitability parameters for Ivabradine and Metoprolol.

S no	Ivabradine			Metoprolol			Resolution	
	Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count		Tailing
1		2.517	11545	1.19	3.097	9143	1.07	4.8
2		2.564	11286	1.19	3.151	9351	1.06	5.1
3		2.566	11029	1.18	3.152	9445	1.07	5.1
4		2.566	11228	1.15	3.153	9609	1.07	5.2
5		2.566	10969	1.17	3.154	9341	1.07	5.2
6		2.566	11198	1.15	3.156	9811	1.06	4.8

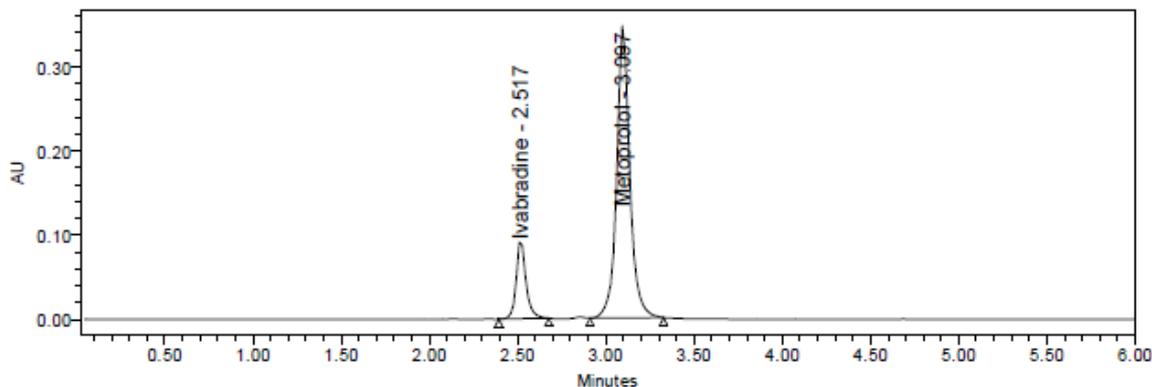


Fig. System suitability Chromatogram.

Validation Specificity

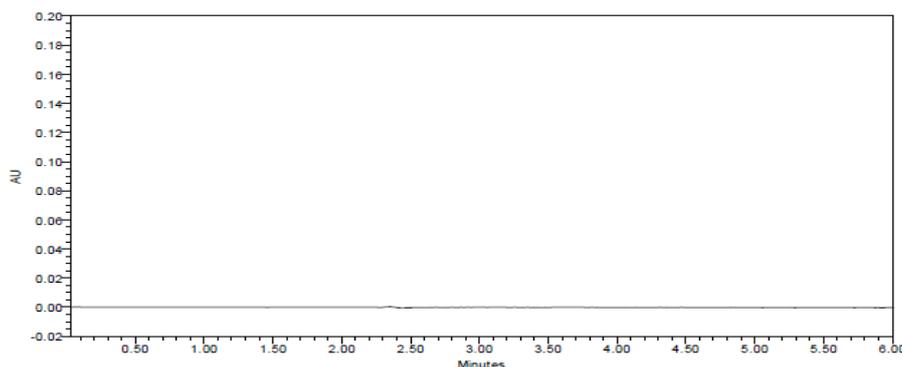


Figure No. 6.12. Chromatogram of blank.



Figure No. Chromatogram of placebo.

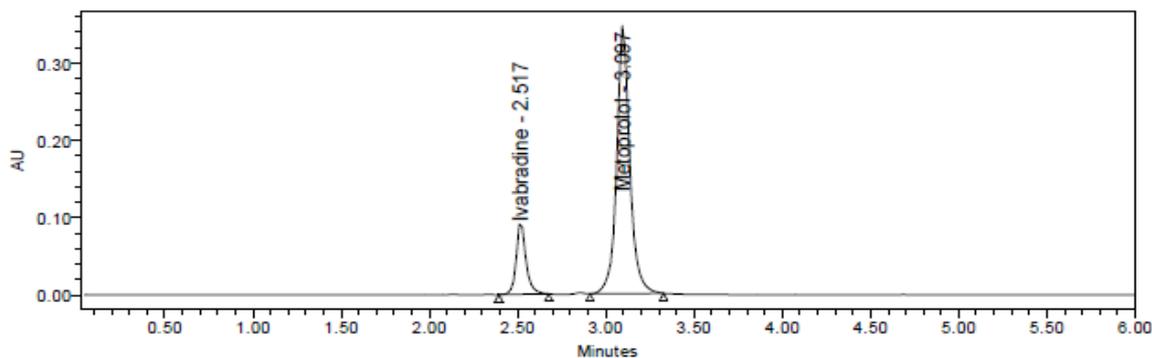


Figure No. Chromatogram of Standard drugs.

Linearity

Table 6.2: Linearity table for Ivabradine and Metoprolol.

Ivabradine		Metoprolol	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
5	89022	25	445716
10	174020	50	870520
15	262132	75	1285318
20	350206	100	1759463
25	435600	125	2177204
30	517582	150	2601428

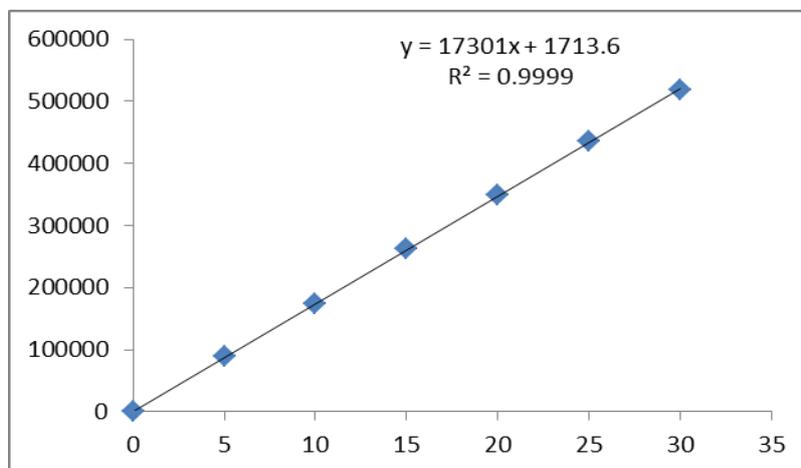


Fig No. 6.15 Calibration curve of Ivabradine.

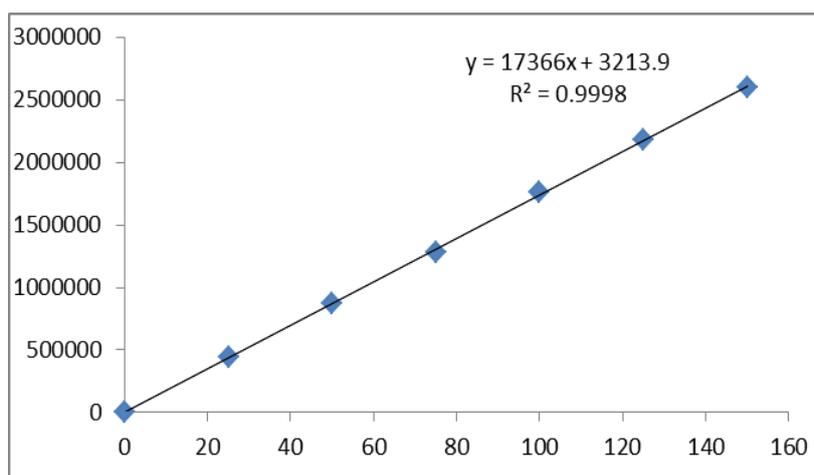


Fig No. 6.16 Calibration curve of Metoprolol.

Precision

System Precision

Table 6.3 System precision table of Ivabradine and Metoprolol.

S. No	Area of Ivabradine	Area of Metoprolol
1.	357442	1783909
2.	360848	1778531
3.	359290	1761279
4.	358201	1777654
5.	352527	1803731
6.	355661	1802373
Mean	357328	1784580
S.D	2927.2	16194.4
%RSD	0.8	0.9

Repeatability**Table 6.4 Repeatability table of Ivabradine and Metoprolol.**

S. No	Area of Ivabradine	Area of Metoprolol
1.	360929	1793038
2.	358499	1780921
3.	362290	1790340
4.	358260	1790090
5.	362119	1782608
6.	356605	1795645
Mean	359784	1788774
S.D	2329.1	5818.3
%RSD	0.6	0.3

Intermediate precision (Day_ Day Precision)**Table 6.5 Intermediate precision table of Ivabradine and Metoprolol.**

S. No	Area of Ivabradine	Area of Metoprolol
1.	345312	1727098
2.	350027	1754114
3.	349898	1741962
4.	348422	1740497
5.	347554	1745818
6.	348020	1747284
Mean	348206	1742796
S.D	1735.5	9053.0
%RSD	0.5	0.5

Accuracy**Table 6.6 Accuracy table of Ivabradine.**

% Level	Amount Spiked ($\mu\text{g/mL}$)	Amount recovered ($\mu\text{g/mL}$)	% Recovery	Mean %Recovery
50%	10	9.9999422	100.00	99.65%
	10	10.044506	100.45	
	10	9.9031848	99.03	
100%	20	20.115485	100.58	
	20	19.92879	99.64	
	20	19.959193	99.80	
150%	30	29.733946	99.11	
	30	29.718109	99.06	
	30	29.745044	99.15	

Table 6.6: Accuracy table of Metoprolol.

% Level	Amount Spiked ($\mu\text{g/mL}$)	Amount recovered ($\mu\text{g/mL}$)	% Recovery	Mean %Recovery
50%	50	50.129	100.26	99.39%
	50	49.829	99.66	
	50	49.821	99.64	
100%	100	100.416	100.42	
	100	100.103	100.10	
	100	100.279	100.28	
150%	150	148.661	99.11	
	150	149.090	99.39	
	150	148.817	99.21	

Sensitivity

Table 6.7 Sensitivity table of Ivabradine and Metoprolol.

Molecule	LOD	LOQ
Ivabradine	0.31	0.95
Metoprolol	0.68	2.06

Robustness

Table 6.8 Robustness data for Ivabradine and Metoprolol.

S.no	Condition	%RSD of Ivabradine	%RSD of Metoprolol
1	Flow rate (-) 0.9ml/min	0.3	0.2
2	Flow rate (+) 1.1ml/min	0.4	0.7
3	Mobile phase (-) 55B:45A	0.6	0.5
4	Mobile phase (+) 45B:55A	1.2	1.2
5	Temperature (-) 25°C	1.0	0.6
6	Temperature (+) 35°C	0.3	0.6

Assay: Intas Pharmaceuticals Ltd (IVA Met), bearing the label claim Ivabradine 5mg, Metoprolol 25mg. Assay was performed with the above formulation. Average %

Assay for Ivabradine and Metoprolol obtained was 100.28% and 100.03% respectively.

Table 6.9 Assay Data of Ivabradine.

S.no	Standard Area	Sample area	% Assay
1	357442	360929	100.60
2	360848	358499	99.93
3	359290	362290	100.98
4	358201	358260	99.86
5	352527	362119	100.94
6	355661	356605	99.40
Avg	357328	359784	100.28
Stdev	2927.2	2329.1	0.65
%RSD	0.8	0.6	0.6

Table 6.10 Assay Data of Metoprolol.

S. no	Standard Area	Sample area	% Assay
1	1783909	1793038	100.27
2	1778531	1780921	99.60
3	1761279	1790340	100.12
4	1777654	1790090	100.11
5	1803731	1782608	99.69
6	1802373	1795645	100.42
Avg	1784580	1788774	100.03
Stdev	16194.4	5818.3	0.3
%RSD	0.9	0.3	0.3

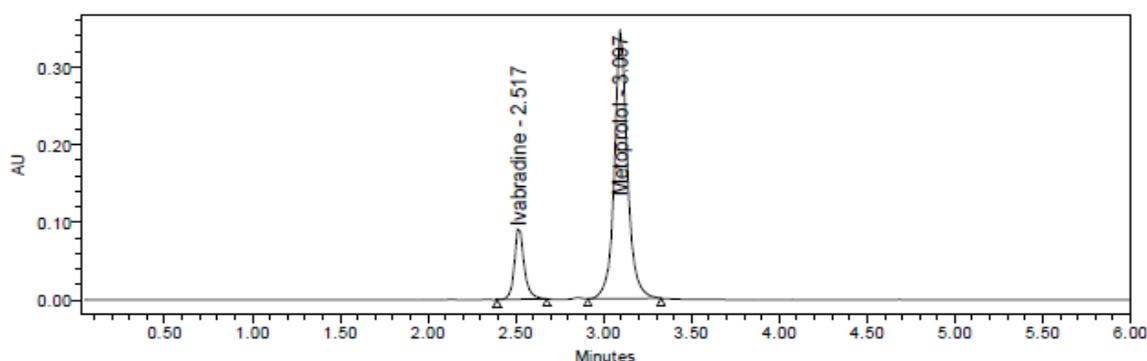


Fig. Chromatogram of working standard solution.

6.8. Degradation data.

Type of degradation	Ivabradine			Metoprolol		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	332482	92.67	7.33	1661205	92.90	7.10
Base	340629	94.95	5.05	1689240	94.47	5.53
Peroxide	335706	93.57	6.43	1705371	95.37	4.63
Thermal	349197	97.33	2.67	1747364	97.72	2.28
Uv	354448	98.80	1.20	1767841	98.86	1.14
Water	356146	98.80	1.20	1772063	99.10	0.90

SUMMARY AND CONCLUSION

Parameters	Ivabradine	Metoprolol	LIMIT	
Linearity Range ($\mu\text{g/ml}$)	5-30 $\mu\text{g/ml}$	25-150 $\mu\text{g/ml}$	R < 1	
Regression coefficient	0.999	0.999		
Slope(m)	17301	17366		
Intercept(c)	1713	3213		
Regression equation ($Y=mx+c$)	$y = 17301x + 1713.$	$y = 17366x + 3213$		
Assay (% mean assay)	100.28%	100.03%	90-110%	
Specificity	Specific	Specific	No interference of any peak	
System precision %RSD	0.8	0.9	NMT 2.0%	
Method precision %RSD	0.6	0.3	NMT 2.0%	
Accuracy %recovery	99.65%	99.39%	98-102%	
LOD	0.31	0.68	NMT 3	
LOQ	0.95	2.06	NMT 10	
Robustness	FM	0.3	0.2	%RSD NMT 2.0
	FP	0.4	0.7	
	MM	0.6	0.5	
	MP	1.2	1.2	
	TM	1.0	0.6	
	TP	0.3	0.6	

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivabradine and Metoprolol in Tablet dosage form. Retention time of Ivabradine and Metoprolol were found to be 2.517min and 3.097min. %RSD of the Ivabradine and Metoprolol were found to be 0.6 and 0.3 respectively. %Recovery was obtained as 99.65% and 99.39 % for Ivabradine and Metoprolol respectively. LOD, LOQ values obtained from regression equations of Ivabradine and Metoprolol were 0.31, 0.95 and 0.68, 2.06 respectively. Regression equation of Ivabradine is $y = 17301x + 1713.$, and $y = 17366x + 3213$ of Metoprolol. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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