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Stability indicating RP-HPLC method development and validation for simultaneously estimation of ivabradine and metaprolol in bulk and pharmacetical dosage form

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ABSTRACT

A Stable, selective, easy and accurate 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 µm. phosphate buffer and Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1.0 ml/min. 260nm selected as optimum wavelength. 2.517min and 3.097min are retention time of Ivabradine and Metoprolol. 99.65% and 99.39 % recovery values for Ivabradine and Metoprolol respectively. 0.31, 0.95ppm and 0.68, 2.06 are LOD and LOQ values for Ivabradine and Metoprolol. 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.

Keywords: Ivabradine, Metoprolol, RP-HPLC

INTRODUCTION

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 (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 [11]. 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.

Mechanism of action

Ivabradine brings down pulse by specifically restraining if channels ("clever channels") in the heart in a focus subordinate way without influencing some other cardiovascular ionic

channels (counting calcium or potassium). Ivabradine ties by entering and joining to a site on the channel pore from the intracellular side and disturbs If particle current stream, which delays diastolic depolarization, bringing down pulse. Ivabradine along these lines brings down the pacemaker terminating rate, thusly bringing down pulse and diminishing myocardial oxygen request. This takes into account an enhanced oxygen supply and hence moderation of ischemia, considering a higher exercise limits and decreases in angina scene [1].

Metoprolol

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].

Mechanism of action

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Literature survey revealed that there were few analytical methods have been reported for Ivabradine and Metoprolol in LC MS/MS has been reported.

However, an extensive literature search didn't reveal any estimation method for Ivabradine and Metoprolol in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for estimation of Ivabradine and Metoprolol in API and Pharmaceutical dosage form [3-8].

MATERIALS AND METHODS

Chemicals and Reagents

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. All active pharmaceutical ingredients (APIs) of Ivabradine and Metoprolol as reference standards were procured from Spectrum Pharma labs, Hyderabad, India [9-12].

Instruments and Chromatographic Conditions

Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Acuity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Ivabradine and Metoprolol solution. The mobile phase used was 0.1% orthophosphoric acid: Acetonitrile (45:55A) at a flow rate of 1ml/min, samples were analyzed at 234 nm detector wave length and at an injection volume of 10 µL using discovery C_{18} 150 x 4.6 mm, 5µ with run time of 5 min.

METHODS
OPTIMIZED METHOD

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

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.

Standard Preparation

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).

SAMPLE PREPARATION

Preparation of Standard 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).

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)

METHOD VALIDATION

Proper validation of analytical methods is important for pharmaceutical analysis when ensure of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria.

Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the international conference on harmonization (ICH) guidelines (Q2A and Q2B). The US food and drug administration (FDA) and US Pharmacopoeia (USP) both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantitation limit, linearity, range, robustness and stability of analytical solutions. Method validation must have a written and approved protocol prior to use.

Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

Linearity

The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data is then processed using a linear least-squares regression. The resulting plot slope, intercept and correlation coefficient provide the desired information on linearity.

Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies. There are three ways to determine accuracy.

1. Comparison to a reference standard
2. Recovery of the analyte spiked into blank matrix or
3. Standard addition of the analyte.

It should be clear how the individual or total impurities are to be determined. e.g.,Weight / weight or area percent in all cases with respect to the major analyte.

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 1and 2. (200µg/ml of Ivabradine and 1000µg/ml of Metoprolol)

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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)

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". A good practice is to vary important parameters in the method systematically and measure their effect on separation. The variable method parameters in HPLC technique may involves flow rate, column temperature, sample temperature, pH and mobile phase composition.

LOD sample Preparation

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily qualtitated, under the stated experimental conditions. With UV detectors, it is difficult to assure the detection precision of low level compounds due to potential gradual loss of sensitivity of detector lamps with age or noise level variation by detector manufacturer. At low levels, assurance is needed that the LOD and LOQ limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability,extraneous peak(s) could "disappear / appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for LOD from the area counts of the analyte. Several approaches for determining the LOD are possible, depending on whether the procedure is a non-instrumental or instrumental.

LOQ sample Preparation

Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Several approaches for determining the LOQ are possible depending on whether the procedure is a non-instrumental or instrumental.

System suitability parameters

Prior to the analysis of samples of each day, the operator must establish that the HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and Precision. The requirements for system suitability are usually developed after method development and validation have been completed.

Assay of Ivabradine and Metoprolol

An Accurately measured weight equivalent to (**IVAMET XL**) 25mg of Ivabradine and Metoprolol was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the HPLC system and scanned at 234 nm from which the % of drug was estimated.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions

To develop and establish a suitable RP-HPLC method for estimation of Ivabradine and Metoprolol in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 45% Ortho phosphoric acid:55% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 234 nm detector

wave length and at an injection volume of 10 µL using Discovery C18 4.6 x 250mm, 5µm.with run time of 5 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Ivabradine and Metoprolol, the optimized chromatogram was obtained as shown in (Figure-2).

Validation

Linearity was established (18.75-112.5µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 15456x + 2384, correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-3). Retention times of Ivabradine and Metoprolol were 2.567min. Where no interfering peaks in blank and placebo at retention time of this drug was not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 99.00% was shown in (Table-2).% RSD was calculat ed from the corresponding peaks obtained by injecting six times a known concentration of Ivabradine and Metoprolol was obtained as 0.6% and the % RSD for intermediate Precision was obtained as 0.7%, Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Ivabradine and Metoprolol . The detection limit values were obtained as 0.085 and Quantitation limit were fund to be 0.258 as given in (Table-4).Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (50:50) mobile phase plus (40:60) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner Table -5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Ivabradine and Metoprolol pure drugs (API) was obtained from spectrum Pharma research solutions and pfizer Ltd (Adriamycin), bearing the label claim 75mg. Assay was performed with the above formulation. Average % Assay obtained was 98.82% the result was shown in (Table-7) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-4, 5) respectively [14-23].

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8).

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 and 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 [24-26].

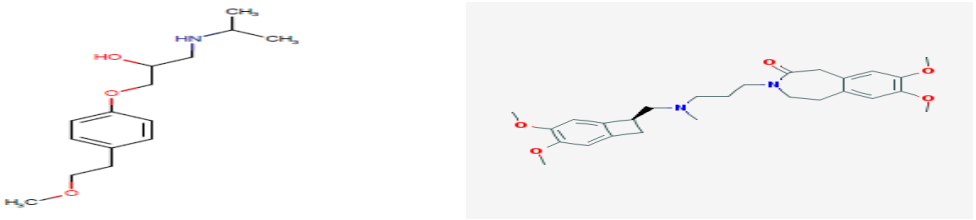


Figure-1: Chemical Structure of Ivabradine and Metoprolol

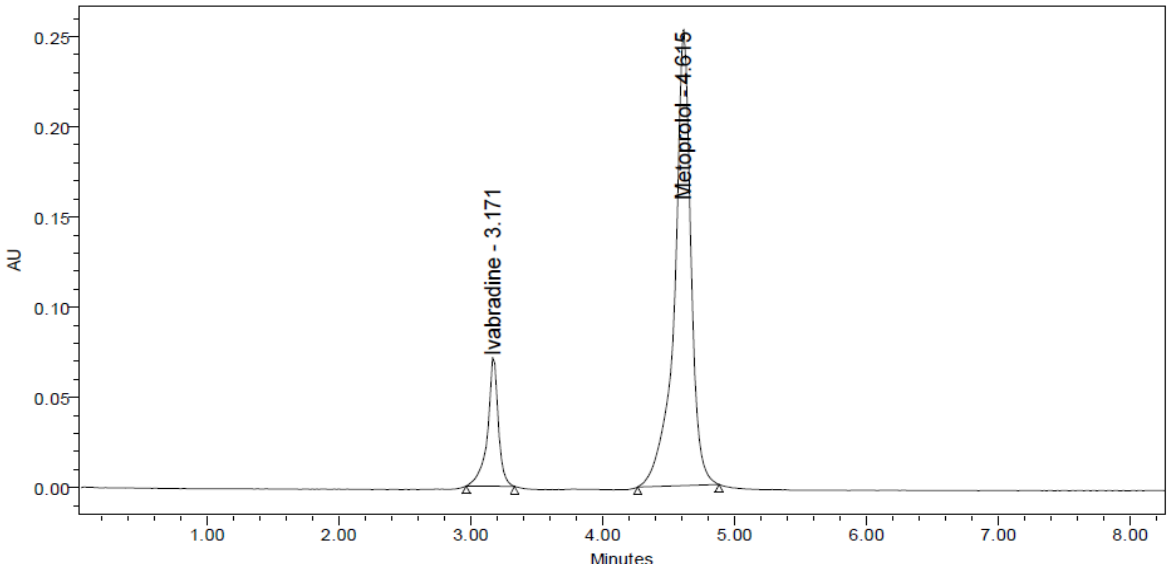


Figure-2: Optimized Chromatogram of Ivabradine and Metoprolol

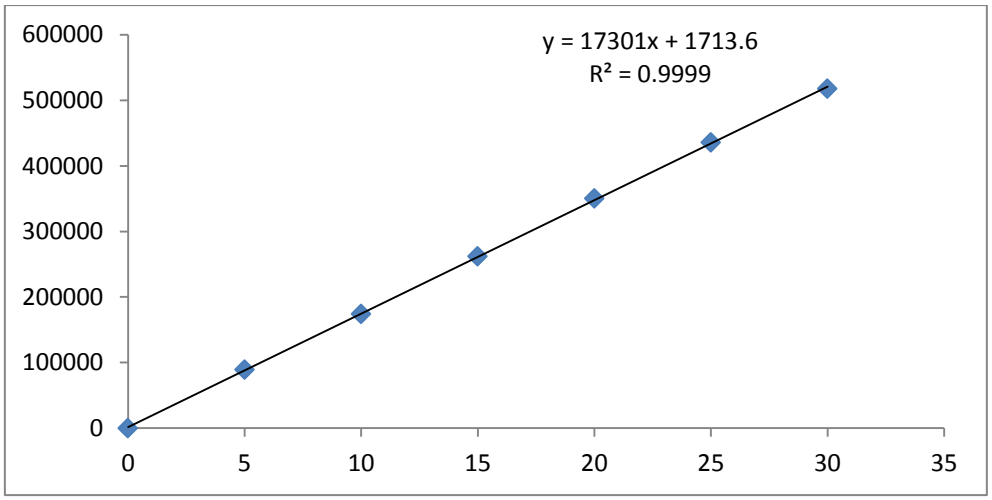
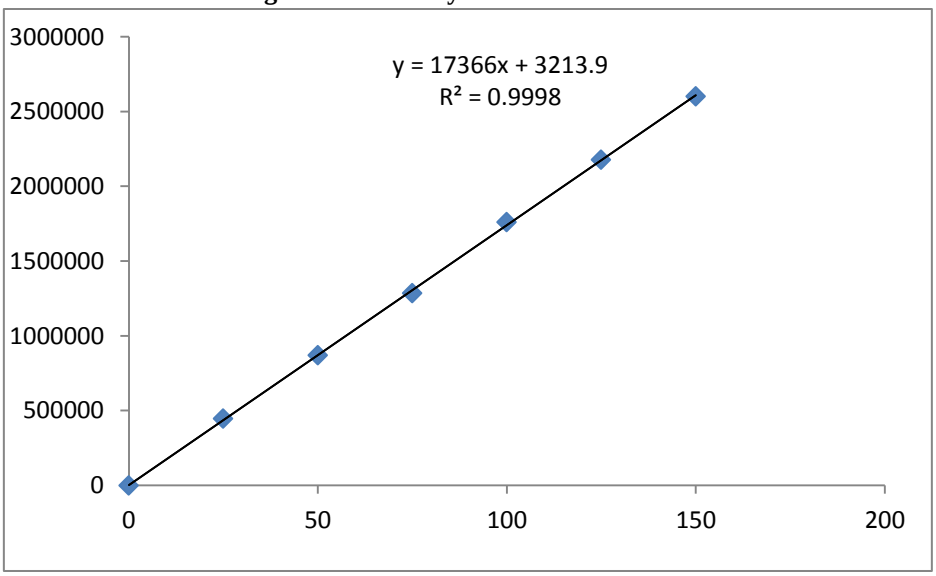


Figure-3: Linearity Curve of Ivabradine



Calibration curve of Metoprolol

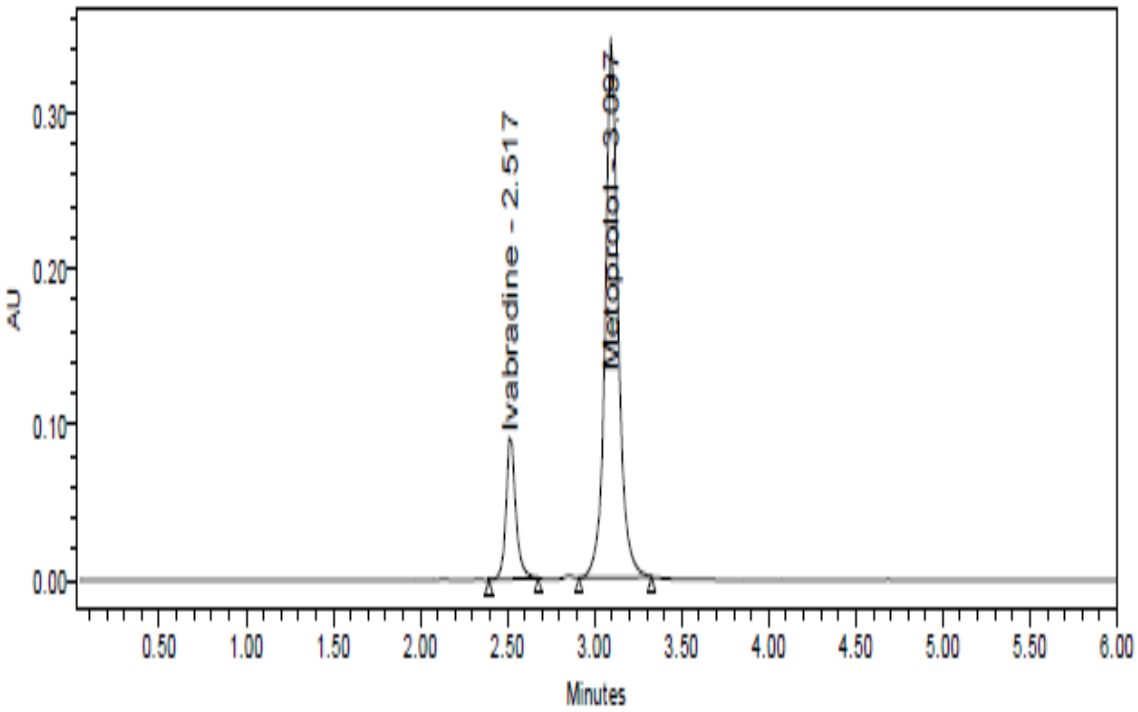


Figure-4: Standard Chromatogram of Ivabradine and Metoprolol

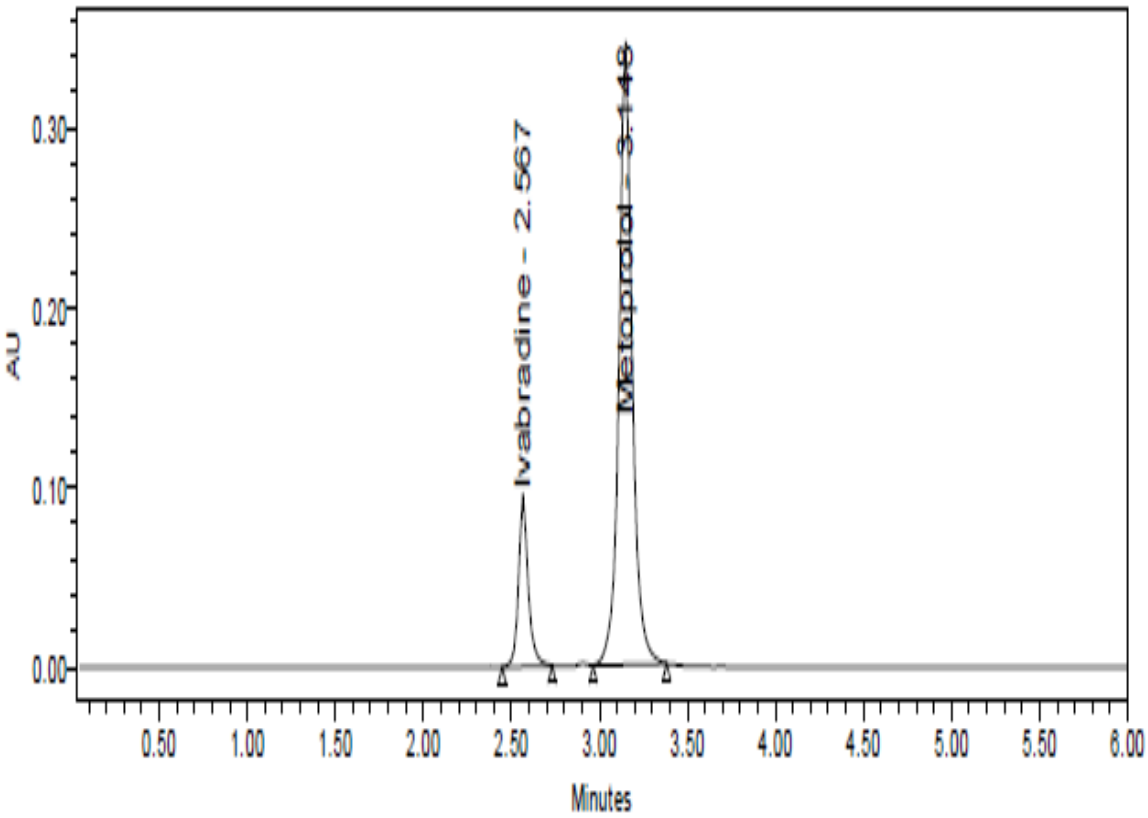


Figure-5: A Sample Chromatogram of Ivabradine and Metoprolol in Pharmaceutical

Dosage FormTable-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	KH2PO4: Acetonitrile (55:45)
Flow rate	1.0 ml/min
Column	Discovery C8 250mm x 4.6 mm, 5µm.
Detector wave leng	PDA 260nm
Column temperatu	30°C
Injection volume	10µL
Run time	10.0 min
Diluent	Water and Acetonitrile in the 50:50 Ratio
Retention Time	Ivabradine (2.517min) and Metoprolol and (3.097mi
Theoretical Plates	Ivabradine (10632.4)and Metoprolol (9510.0)

Accuracy table of Ivabradine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/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-2: Accuracy results of Metoprolol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/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	

Table-3: Precision Result 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

Table-4: LOD and LOQ values of Ivabradine and Metoprolol and Elbasvir

Molecule	LOD	LOQ
Ivabradine	0.31	0.95
Metoprolol	0.68	2.06

Table-5 Robustness Data of 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

Table-6: System Suitability Parameters Result of Ivabradine and Metoprolol

S no	Ivabradine			Metoprolol			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
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

Table -7: Assay Results 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

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

Table 8. Degradation Data of Ivabradine and Metoprolol

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

REFERENCE

[1]. R. S. Satoskar, S. D. Bhandarkar and S. S. Ainapure. "Pharmacology and Pharmacotherapeutics", 17th edition, Popular Prakashan, Mumbai, India, 2001.

[2]. "Burger's Medicinal Chemistry and drug discovery", 6 th edition, Wiley Interscience, New Jersey, 2007.

[3]. "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", Lippincott Williams & Wilkins, New york, 11, 2004.

[4]. A. Korolkovas. "Essentials of Medicinal Chemistry", 2nd edition, Wiley Interscience, New Jersey, 1988.

[5]. "Goodman and Gilman's The Pharmacological Basis of Therapeutics", McGraw-Hill health professions division, New york, 9, 1996.

[6]. Foye's "Principles of Medicinal Chemistry", Lippincott Williams & Wilkins, New york, 6, 2008.

[7]. Drugs & Cosmetics Act, 1940 & Rules, 1945, Susmit publishers, Mumbai, India, 2, 2000.

[8]. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.

[9]. The United States Pharmacopoeia- the National Formulary, United States Pharmacopoeial convention, Rockville, 2007.

[10]. British Pharmacopoeia, The Stationary Office, London, 2005.

[11]. "Martindale - The Extra Pharmacopoeia", 33rd edition, The Pharmaceutical Press, London, 2002. 7

[12]. A. H. Beckett and J. B. Stenlake. "Practical Pharmaceutical Chemistry", CBS Publishers & Distributors, New Delhi, India, 1,2, 2000.

[13]. P. D. Sethi. "Quantitative Analysis of Drugs in Pharmaceutical Formulations". CBS Publishers & Distributors, New Delhi, India, 3, 1997.

[14]. H. H. Willard, L. L. Merrit, J. A. Dean and F. A. Settle. "Instrumental Method of Analysis", 7th edition, CBS Publishers & Distributors, New Delhi, India, 1986.

[15]. R. A. Day and A. L. Underwood. "Quantitative Analysis", PHI learning private limited, New Delhi, India, 6, 2009.

[16]. https://www.drugbank.ca/drugs/DB09083

[17]. https://www.scbt.com/scbt/product/ivabradine-d3-hydrochloride-1217809-61-4

[18]. https://www.drugbank.ca/drugs/DB00264

[19]. https://www.scbt.com/scbt/product/s-metoprolol-81024-42-2

[20]. Sagarika, Panda.Srikanta,Patra. Rapid and Selective UV Spectrophotometric and RP-HPLC Methods for Dissolution Studies of Ivabradine controlledRelease Formulations. *PharmaTutor*, *2(8)*, 2014, 201-213.

[21]. Bhosale Swati, D.;VanjariSuvarna, S.;Jagtap Nikhil, S. Development and validation of stability indicating spectrophotometric method for the estimation of ivabradine hydrochloride in bulk and in tablet formulation. *wjpps*. 5(7), 2016, 1919-1927.

[22]. Sunitha,Seerapu.; Srinivasan,B. P.Development and Validation of RP-HPLC Method for the Estimation of Ivabradine Hydrochloride in Tablets.*Indian J Pharm Sci*, *72(5)*, 2010, 667–671.

[23]. Muzaffar-ur- Rehman,Md.; Nagamallika,G.Validated RP-HPLC Method for the Determination of Ivabradine Hydrochloride in Pharmaceutical Formulation.*Int. J. Pharm. Sci. Drug Res*. *9(5)*, 2017, 228-233.

[24]. Mahaparale,S.I; Gonjari,D.; Jaya veera,K.N. Stability indicating hplc method for simultaneous estimation of metoprolol succinate and telmisartan.*Journal of Liquid Chromatography & Related Technologies*. *36(18)*, 2013, 2601-2611.

[25]. Selvakumar.;Pandiyan.; Rajagopal. Development and validation of stability indicating rapid hplc method for estimation of ivabradine hydrochloride in solid oral dosage form.*IJPPS*, 6*(4)*, 2014, 378-382.

[26]. Naveen Kumar,C.H.;SaraswathiMannuri.;VijayaKuchana.; Kannappan,N. Development and validation of a stability indicating RP-HPLC method for determination of metoprolol succinate in pharmaceutical dosage forms.*Der Pharmacia Sinica*, *5(6)*, 2014, 69-78.