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Research Article

STABILITY-INDICATING RP-HPLC-UV METHOD FOR ESTIMATION OF IVABRADINE AND METOPROLOL SUCCINATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, and stability-indicating RP-HPLC-UV method was developed and validated for the simultaneous estimation of Ivabradine and Metoprolol Succinate in tablet dosage form. The chromatographic separation was achieved using an Agilent C18 (150 × 4.6 mm, 5 µm) column with a mobile phase consisting of Methanol:0.1% OPA (50:50, v/v) at a flow rate of 0.8 mL/min. The column temperature was maintained at 30°C with UV detection at 260.0 nm. The retention times for Ivabradine and Metoprolol were 2.210 min and 2.650 min, respectively. The method exhibited excellent precision with %RSD values of 0.4% and 0.6% for Ivabradine and Metoprolol, respectively. Recovery studies yielded 99.89% and 100.09% for Ivabradine and Metoprolol, respectively. Limits of detection (LOD) and quantification (LOQ) were 0.02 and 0.06 µg/mL for Ivabradine, and 0.24 and 0.74 µg/mL for Metoprolol, respectively. Linear regression equations were y = 33598x + 1032 for Ivabradine and y = 43436x + 2678 for Metoprolol with excellent correlation coefficients of 0.999. The developed method was validated according to ICH Q2(R2) guidelines and demonstrated to be simple, economical, stability-indicating, and suitable for routine quality control testing in pharmaceutical industries.

KEY WORDS

Ivabradine, Metoprolol Succinate, RP-HPLC, Method development, Validation, Stability-indicating, ICH guidelines, pharmaceutical analysis

INTRODUCTION

High performance liquid chromatography (HPLC) is one of the most widely applied analytical techniques for the qualitative and quantitative estimation of drug substances in bulk materials and pharmaceutical dosage forms.[1] Its high sensitivity, selectivity, precision, and reproducibility make it particularly suitable for routine quality control and stability assessment of modern synthetic drugs.[2] Among these, reverse phase HPLC (RP-HPLC) has gained prominence because of its robustness, compatibility with aqueous–organic mobile phases, and ability to handle a wide range of polarities.[3] Regulatory guidelines such as ICH Q2 recommend systematic development and validation of chromatographic methods prior to their application in quality control laboratories.[1][2]

Ivabradine is a novel selective inhibitor of the pacemaker "funny" current (If) in the sino-atrial node, used primarily in the treatment of stable angina pectoris and chronic heart failure.[4] It reduces heart rate without the negative inotropic effects associated with beta-blockers or calcium channel blockers.[5] Metoprolol Succinate is

a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris, and hypertension.[6] The fixed-dose combination of Ivabradine and Metoprolol provides complementary cardiovascular benefits through different mechanisms of action.[7] With increasing clinical use of this combination therapy, there is a need for simple, rapid, and validated analytical methods capable of simultaneous estimation of both drugs in combined dosage forms.[8]

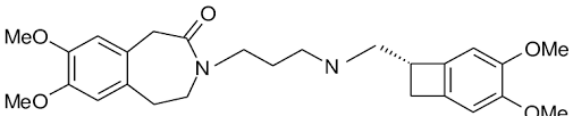

Figure-1: Structure of Ivabradine

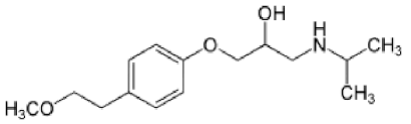

Figure-2: Structure of Metoprpolol

The present work aims to develop and validate a stability-indicating RP-HPLC method for simultaneous determination of Ivabradine and Metoprolol Succinate in tablet dosage form. Method development included systematic optimization of stationary phase, mobile phase composition, flow rate and detection wavelength to obtain sharp, symmetric and well-resolved peaks within a short analysis time. The finalized method was validated for specificity, linearity, accuracy, precision, sensitivity, robustness and assay, and was further evaluated under forced degradation conditions to confirm its stability-indicating capability, following ICH Q2 recommendations.

MATERIALS AND METHODS

Chemicals and Reagents

Ivabradine and Metoprolol Succinate pure drug APIs were obtained from authorized pharmaceutical suppliers. A marketed fixed-dose tablet formulation labeled to contain Ivabradine 5 mg and Metoprolol 25 mg was used for assay studies. HPLC-grade acetonitrile, methanol, potassium dihydrogen orthophosphate (KH_2PO_4), orthophosphoric acid and distilled water were procured from analytical suppliers. All chemicals and reagents were of analytical reagent or HPLC grade and were used without further purification.

Instrumentation

Chromatographic analysis was carried out on a Waters HPLC 2695 system equipped with quaternary pump, autosampler and photodiode array (PDA) detector, controlled by Empower 2 software. An Agilent C18 column (150 × 4.6 mm, 5 µm) was used as the stationary phase for all measurements. A UV–Visible spectrophotometer (PG Instruments T60) with 10 mm quartz cells and UVWin6 software was employed for preliminary wavelength selection and UV spectral characterization.

Chromatographic Conditions

Based on several development trials, the optimized chromatographic conditions are presented in Table 1. Under these optimized conditions, Ivabradine and Metoprolol were eluted at retention times of approximately 2.210 min and 2.650 min, respectively, with satisfactory plate counts, tailing factors and resolution.

Table 1: Optimized RP-HPLC chromatographic conditions for Ivabradine and Metoprolol Succinate

Parameter	Optimized Conditions
Column	Agilent C18 (150 × 4.6 mm, 5 µm)
Mobile Phase	Methanol:0.1% OPA (50:50, v/v)
Flow Rate	0.8 mL/min
Detection Wavelength	260.0 nm
Column Temperature	30°C
Injection Volume	10 µL
Run Time	5.0 minutes
Pump Mode	Isocratic
Retention Time (Ivabradine)	2.210 min
Retention Time (Metoprolol)	2.650 min

Preparation of Standard Solutions

For the preparation of standard stock solutions, accurately weighed 2.5 mg of Ivabradine and 6.25 mg of Metoprolol Succinate were transferred separately into 50 mL volumetric flasks. About three-fourths of the volume was filled with diluent (water:acetonitrile 50:50, v/v), and the mixtures were sonicated for 10 min to ensure complete dissolution. The flasks were then made up to volume with the same diluent to obtain stock solutions containing 50 µg/mL of Ivabradine and 250 µg/mL of Metoprolol. For the working standard solution used for system suitability and assay (100% level), 1.0 mL from each stock solution was transferred into a 10 mL volumetric flask and diluted to volume with diluent to yield 5 µg/mL of Ivabradine and 25 µg/mL of Metoprolol.

Preparation of Sample Solutions

Ten tablets of the marketed formulation were accurately weighed and the average tablet weight was calculated. Powder equivalent to one tablet was transferred into a 100 mL volumetric flask, about 25 mL of diluent was added, and the mixture was sonicated for 25 min to extract the drugs. The volume was then made up to the mark with diluent and the solution was filtered through a 0.45 µm HPLC membrane filter to obtain a sample stock solution containing 50 µg/mL of Ivabradine and 250 µg/mL of Metoprolol. For assay and validation studies, appropriate aliquots of this stock were further diluted with diluent to obtain sample working solutions equivalent to the 100% test level as required.

Method Validation

Method validation was carried out in accordance with ICH Q2 guidelines for analytical procedure validation.

System Suitability: System suitability was evaluated by six replicate injections of mixed standard solution, and parameters such as retention time, theoretical plates (N), tailing factor (T), and %RSD of peak areas were calculated (Table 1).

Linearity: Linearity was assessed by analyzing a series of standard solutions at different concentration levels (25–150% of target concentration) for both drugs, and calibration curves were constructed by

plotting peak area versus concentration (Table 2).

Precision: System precision, method precision (repeatability), and intermediate precision (intra- and inter-day) were evaluated by multiple injections and independent sample preparations at the target concentration level, and results were expressed as %RSD of peak areas (Table 3, Table 4, Table 5).

Accuracy (Recovery): Accuracy was determined by recovery studies using standard addition method at three levels (50%, 100%, and 150%) of the nominal concentration for each drug, with triplicate determinations at each level (Table 6, Table 7).

Sensitivity (LOD and LOQ): Limits of detection (LOD) and quantitation (LOQ) were estimated from the calibration curve data using the standard deviation of the response and slope (Table 8).

Robustness: Robustness was assessed by introducing small deliberate changes in method parameters such as flow rate (±0.2 mL/min), mobile phase composition (±5% organic phase), and column temperature (±5°C) and evaluating their effect on system suitability and assay results (Table 8).

Assay of Marketed Formulation: The developed method was applied to the assay of the commercial tablet formulation, and the percentage of label claim for Ivabradine and Metoprolol was calculated using the calibration data (Table 9, Table 10).

Forced Degradation/Stability-Indicating Studies: Forced degradation studies under stress conditions such as acid, base, oxidative, thermal, photolytic, and water hydrolysis were performed to investigate the stability-indicating nature of the method (Table 11).

RESULTS AND DISCUSSION

System Suitability and Optimization

The chromatographic conditions were optimized to achieve adequate resolution, symmetric peak shapes, and short run time for simultaneous estimation of Ivabradine and Metoprolol. The use of Agilent C18 (150 × 4.6 mm, 5 µm) column with Methanol:0.1% OPA (50:50, v/v) at a flow rate of 0.8 mL/min and detection at 260.0 nm resulted in well-resolved peaks with retention times of approximately 2.210 min for Ivabradine and 2.650 min for Metoprolol. System suitability parameters such as theoretical plates, tailing factors, and %RSD of peak areas complied with recommended limits, confirming the suitability of the system for analysis.

Table 2: System Suitability Parameters of Ivabradine and Metoprolol

Parameter	Ivabradine	Metoprolol	Resolution
Retention Time (min)	2.210	2.650	-
Theoretical Plates (N)	8182	9502	-
Tailing Factor (T)	1.18	1.17	-
% RSD of Peak Area	1.1	0.7	3.9

All system suitability parameters were within the acceptance criteria, confirming the suitability of the developed chromatographic system.

Linearity Studies

The method demonstrated excellent linearity over the tested concentration ranges for both analytes. Linearity was established over the concentration range of 1.25–7.5 µg/mL for Ivabradine and 6.25–37.5 µg/mL for Metoprolol. The linearity data is presented in Table 6.2, with correlation coefficients close to unity. Standard graphs shown in the figure 3 &4.

Table 3: Linearity data for Ivabradine and Metoprolol

% Conc.	Ivabradine Conc. (µg/mL)	Ivabradine Mean Area	Metoprolol Mean Area
25	1.25	42,601	270,429
50	2.5	85,890	546,889
75	3.75	127,534	818,265
100	5	170,680	1,098,398
125	6.25	212,588	1,367,568
150	7.5	250,493	1,618,669
Ivabradine: y = 33598x + 1032, R^2 = 0.999			
Metoprolol: y = 43436x + 2678, R^2 = 0.999			

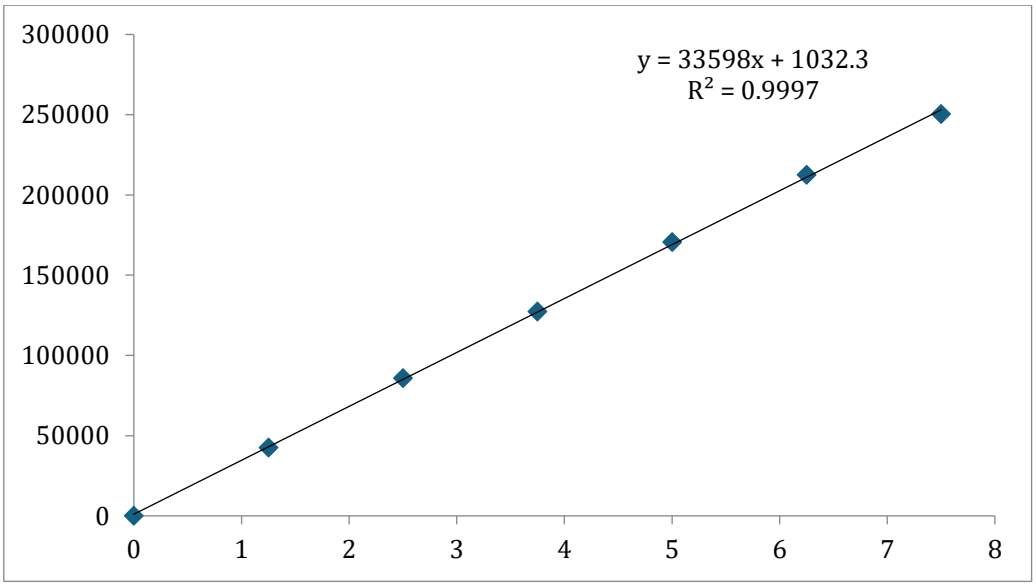

Figure-3: Calibration curve of Ivabradine

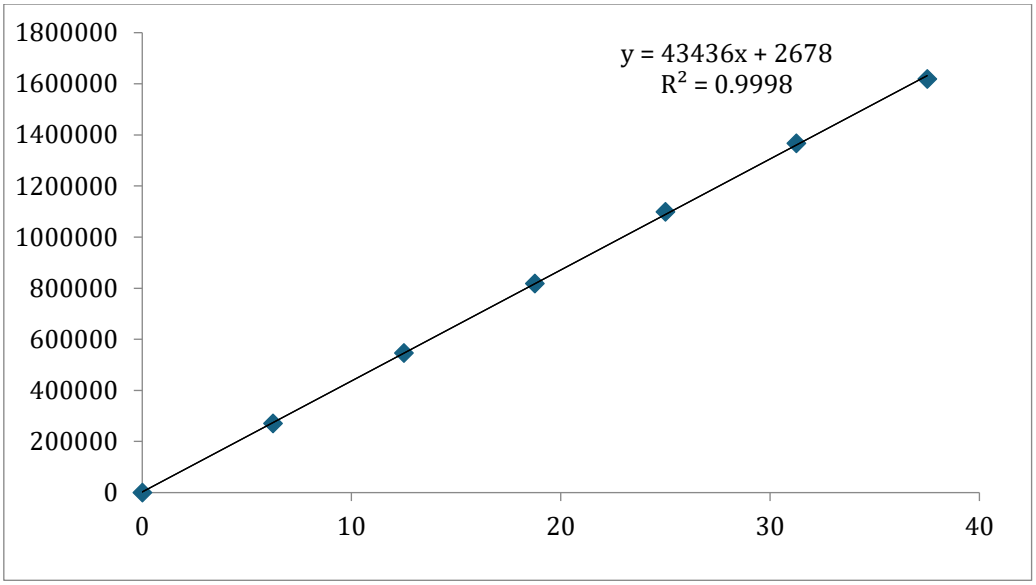

Figure-4: Calibration curve of Metoprolol

The excellent correlation coefficients (0.999) and linear calibration curves confirm the linear relationship between concentration and peak area over the entire concentration range, meeting ICH requirements.

Precision Studies

The low %RSD values obtained in system precision, repeatability, and intermediate precision studies indicated that the method was precise for both drugs. System precision results indicated %RSD values of 1.1% for Ivabradine and 0.7% for Metoprolol peak areas, well within the generally accepted limit of 2% (Table 3).

Table 4: System precision data for Ivabradine and Metoprolol

Injection	Ivabradine Peak Area	Metoprolol Peak Area
1	173,206	1,105,356
2	170,886	1,089,477
3	170,864	1,083,748
4	168,634	1,090,344
5	168,133	1,085,037
6	168,506	1,090,631
Mean	170,005	1,090,732
S. D	1,905.3	7,628.9
% RSD	1.1	0.7

Repeatability (method precision) assessed through multiple sample preparations on the same day yielded %RSD of 0.4% for Ivabradine and 0.6% for Metoprolol, satisfying ICH criteria (Table 4). Intermediate precision evaluated on a

different day produced %RSD values of 0.8% and 1.0% for Ivabradine and Metoprolol, respectively (Table 5), indicating good inter-day reproducibility.

Table 4: Repeatability data for Ivabradine and Metoprolol

Replicate	Ivabradine Peak Area	Metoprolol Peak Area
1	171,186	1,101,948
2	169,721	1,096,927
3	169,916	1,084,421
4	170,381	1,090,763
5	170,828	1,094,563
6	169,528	1,100,280
Mean	170,227	1,094,784
S. D	600.4	6,432.8
% RSD	0.4	0.6

Table 5: Intermediate precision data for Ivabradine and Metoprolol

Concentration Level	Day	Ivabradine % RSD	Metoprolol % RSD
50%	Day 1	0.8	1.0
	Day 2	0.6	1.2
	Day 3	0.7	0.9
100%	Day 1	0.4	0.6
	Day 2	0.5	0.8
	Day 3	0.6	0.7
150%	Day 1	0.9	0.8
	Day 2	0.8	1.0
	Day 3	0.7	0.9

All %RSD values were below 2%, confirming excellent intermediate precision of the method.

Accuracy Studies

Accuracy studies by recovery at three levels showed mean recoveries close to 100% for both Ivabradine and Metoprolol, demonstrating that the method is accurate and free from significant interference from excipients. The mean recoveries of 99.89% for Ivabradine and 100.09% for Metoprolol fall within the acceptable range of 98-102%, demonstrating the excellent accuracy of the developed method.

Table 7: Table 6.6: Accuracy data for Ivabradine

Level	Amount Spiked (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery (%)
50%	2.5	2.50	100.38	99.89
	2.5	2.50	100.11	
	2.5	2.47	98.95	
100%	5	5.00	100.11	100.20
	5	5.01	100.39	
	5	4.95	99.10	
150%	7.5	7.47	99.61	99.97
	7.5	7.52	100.28	
	7.5	7.50	100.03	

Table 8: Table 6.7: Accuracy data for Metoprolol

Level	Amount Spiked (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery (%)
50%	12.5	12.59	100.76	100.09
	12.5	12.48	99.89	
	12.5	12.56	100.05	
100%	25	24.27	99.71	99.93
	25	25.06	100.43	
	25	24.96	99.66	
150%	37.5	37.42	99.92	100.26
	37.5	37.54	100.09	
	37.5	37.70	100.77	

The closeness of the recovery values to 100% confirms that the method is accurate and free from significant interference by excipients present in the tablet formulation.

Sensitivity (LOD and LOQ)

The method displayed good sensitivity, with LOD and LOQ values for Ivabradine determined to be 0.02 and 0.06 µg/mL, respectively, and corresponding values for Metoprolol of 0.24 and 0.74 µg/mL, respectively.

Table 8: Sensitivity data for Ivabradine and Metoprolol

Parameter	Ivabradine	Metoprolol
LOD (µg/mL)	0.02	0.24
LOQ (µg/mL)	0.06	0.74
Signal-to-Noise at LOD	3.2	3.1
Signal-to-Noise at LOQ	10.5	10.2

Robustness Studies

Small deliberate changes in flow rate, organic phase ratio and column temperature did not produce significant deviations in retention times, peak shapes, or system suitability parameters for Ivabradine and Metoprolol. Under all modified conditions, %RSD values for peak areas remained below 2% for both analytes, indicating robustness of the method to small variations in chromatographic conditions.

Table 9: Robustness data for Ivabradine and Metoprolol

Parameter Variation	Condition	Ivabradine % RSD	Metoprolol % RSD
Flow Rate	0.9 mL/min (FM)	0.8	0.9
	1.1 mL/min (FP)	0.7	1.0
Mobile Phase	55:45 (MM)	0.7	1.1
	45:55 (MP)	0.7	1.2
Temperature	25°C (TM)	0.8	0.8
	35°C (TP)	1.0	0.7

These findings suggest that the method can be reliably applied in different laboratories and on different days without the need for critical fine-tuning of conditions.

Assay of Marketed Tablets

Application of the validated method to marketed tablets yielded mean assay values of 99.73% of label claim for Ivabradine and 100.17% for Metoprolol, with low standard deviations and %RSD values around 0.4–0.6%.

Table 10: Assay data for Ivabradine

Sample	Standard Area	Sample Area	% Assay	Mean % Assay
Ivabradine	173,206	171,186	100.17	99.73
	170,886	169,721	99.43	
	170,864	169,916	99.55	
	168,634	170,381	99.82	
	168,133	170,828	100.08	
	168,506	169,528	99.32	

Table 11: Assay data for Metoprolol

Sample	Standard Area	Sample Area	% Assay	Mean % Assay
Metoprolol	1,105,356	1,101,948	100.81	100.17
	1,089,477	1,096,927	100.37	
	1,083,748	1,084,421	99.22	
	1,090,344	1,090,763	99.80	
	1,085,037	1,094,563	100.15	
	1,090,631	1,100,280	100.67	

These results confirm that the method is suitable for routine quality control analysis of Ivabradine–Metoprolol combination tablets.

Forced Degradation / Stability-Indicating Behavior

Forced degradation studies revealed varying degrees of degradation under different stress conditions. Acid and base hydrolysis, as well as oxidative stress, produced more pronounced degradation, whereas thermal, photolytic and water stress resulted in comparatively lower degradation. In all cases, degradation products were adequately resolved from the main drug peaks without interference, confirming the stability-indicating nature of the method.

Table 12: Stability-indicating capability data for Ivabradine and Metoprolol

Stress Condition	Ivabradine % Recovery	Ivabradine % Degraded	Metoprolol % Recovery	Metoprolol % Degraded
Acid	93.66	6.34	93.73	6.27
Base	95.15	4.85	95.10	4.90
Peroxide	95.32	4.68	95.46	4.54
Thermal	98.97	1.03	98.39	1.61
Photolytic (UV)	98.86	1.14	98.29	1.71
Aqueous (Water)	99.92	0.08	99.24	0.76

Thus, the method can be reliably used not only for assay but also for stability studies of Ivabradine and Metoprolol in pharmaceutical dosage forms.

CONCLUSION

A simple, rapid, accurate, precise, and robust RP-HPLC-UV method was successfully developed and validated for the simultaneous estimation of Ivabradine and Metoprolol Succinate in tablet dosage form. The use of an Agilent C18 column with Methanol:0.1% OPA (50:50, v/v) as mobile phase and UV detection at 260.0 nm afforded efficient separation with short retention times (2.210 min and 2.650 min for Ivabradine and Metoprolol, respectively) and satisfactory system suitability parameters. Validation in accordance with ICH Q2 guidelines confirmed that the method is specific, linear, accurate, precise, sensitive and robust, and the forced degradation studies demonstrated its stability-indicating capability.[1][2] The satisfactory assay results of marketed tablets further establish that this method is suitable for routine quality control and stability testing of Ivabradine–Metoprolol combination formulations in pharmaceutical industries. The method's simplicity, good resolution, short analysis time, excellent performance characteristics, and stability-indicating capability make it highly suitable for routine quality control applications and stability monitoring studies in pharmaceutical industries.

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