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A New RP-UPLC Method for Simultaneous Quantification of Ivabradine and Metoprolol in Combined Dosage Forms

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Abstract— *A simple, sensitive, precise, accurate and reportable method was developed for Ivabradine and Metoprolol succinate using a simple phase UPLC system. The chromatographic system consisted of a Shimadzu Nexera XR UPLC chromatograph equipped with a Hibar C18 (100 X 2.1 mm, 2 µ) column, FCV-32AH pumps and an SPD-20A photo diode array (PDA) detector. The mobile phase used was Water and Methanol in the ratio of 70:30. The flow rate was found to be 0.5mL min -1 and the detection wavelength was 260nm. The retention times of Ivabradine and Metoprolol were found to be 1.197 and 1.628min, respectively. The detection limits for Ivabradine and Metoprolol were found to be 0.06 and 0.28 µg mL-1, respectively. The limit of quantification (LOQ) for Ivabradine and Metoprolol was found to be 0.19 and 0.85 µg mL-1, respectively. This method has been validated according to ICH guidelines and can be used to evaluate drug purity in bulk and dosage forms.*

Keywords— *ICH guidelines; Ivabradine; Metoprolol; Methanol; UPLC; Water.*

I. INTRODUCTION

High pressure (up to 100 MPa) and low particle size are the main reasons for the adoption of UPLC in the separation and measurement of analytes. In contrast to HPLC, UPLC's analytical column will not be damaged by the high pressure, even though HPLC's pressure is much lower. UPLC uses less solvent than HPLC since the analytes may be separated in a shorter time period of time. [1-5]

A) Ivabradine

Stable angina pectoris and chronic heart failure may both be treated with Ivabradine, a new heart-rate reducing medication. Patients with chronic heart failure who are not taking beta-blockers owing to contraindications or are already taking the maximal dosage of beta-blockers may now use Ivabradine. [6]

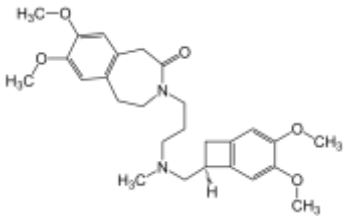

Figure 1. Ivabradine Chemical Structure

B) Metoprolol

The succinate and tartrate derivatives of metoprolol, a selective beta-1 blocker, are extensively used as a formulation for immediate or prolonged release. [7]

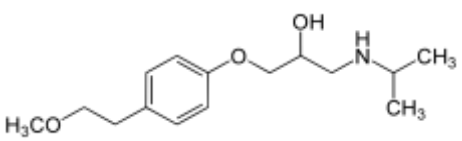

Figure 2. Metoprolol Chemical Structure

II. METHOD AND MATERIALS

Matrix Ltd. provided the reference standard specimens of metoprolol and ivabradine. Acetonitrile and Methanol used was of UPLC grade, while Sodium hydroxide, hydrogen peroxide was of GR grade (Merck Ltd. Mumbai, India). Water from Milli-Q was used for the entire analysis.

A) Preparation of the movable phase

Water and methanol were mixed in the ratio of 70:30 v/v and sonicated to degas.

B) Diluent Preparation

To prepare medication solutions, water and Acetonitrile were combined in the ratio of 50:50 percent v/v.

C) Preparation of the mixed working standard solution of Ivabradine and Metoprolol

Weighing precisely, 5 mg of ivabradine and 25 mg of metoprolol were added to a 100 mL dry volumetric flask. 50 mL of the diluent was added to it and sonicated for 5 min. The final volume was made up with the diluent. This solution contains 50µg/mL of Ivabradine and 250 µg/mL of Metoprolol.

5 mL from the above stock solutions was taken into a 50 mL volumetric flask and diluted to 50 mL to get a working standard solution containing 5 µg/mL of Ivabradine and 25 µg/mL of Metoprolol.

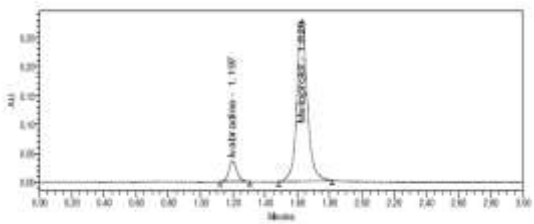

Figure 3. Chromatogram of the mixed standard solution of Ivabradine and Metoprolol

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Under the above optimized conditions, the retention times obtained for Ivabradine and Metoprolol were 1.197 and 1.628 min respectively.

III. METHOD VALIDATION

Validation of the proposed technique was carried out in accordance with ICH recommendations to ensure that it satisfied all of the required criteria, including system appropriateness, linearity, accuracy, precision and robustness, as well as limit of detection and limit of quantification.

A) System Suitability

Ivabradine and Metoprolol were combined and tested to determine the system's applicability. The chromatographic parameters (resolution, theoretical plates, tailing factor, elution time, etc.).

TABLE 1: System suitability values for the present technique

	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

B) Specificity

Checking for any interference peaks in the formulation samples is one way to ensure that the method is specific for the analyte of interest. The method's specificity was assessed in light of potential interference from excipients. Because the excipients utilized in the formulation had no effect on the peak concentrations of the drugs, the procedure may be considered precise. No interference was found in the drug matrix HPLC chromatograms generated for the drug and the excipient combination.

C) Linearity

To establish the linearity, a stock solution containing 125 µg/mL Ivabradine and 625 µg/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 triplicate by injecting 2 µL into the HPLC system. Linearity data for Ivabradine and Metoprolol are given in the table 2 and the corresponding Linearity plots are depicted in Fig. 4 & 5 respectively.

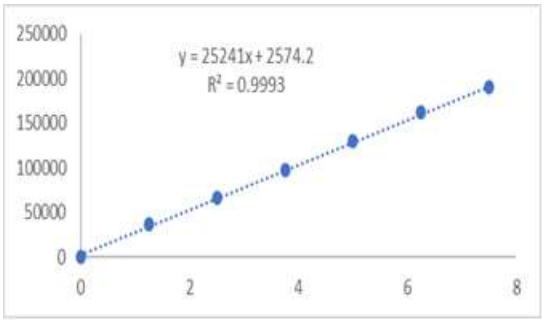

Figure 4. =Linearity Plot of Metoprolol

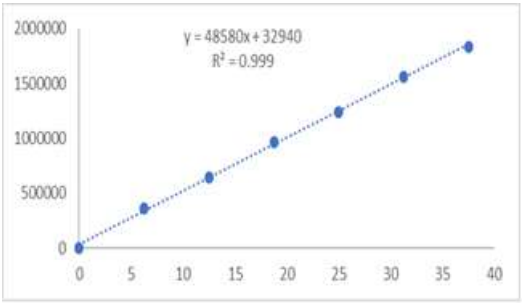

Figure 5. Linearity Plot of Ivabradine

TABLE 2: Linearity of Ivabradine and Metoprolol

Ivabradine		Metoprolol	
Conc (µg/ml)	Mean Area	Conc (µg/ml)	Mean Area
1.25	36618	6.25	361744
2.5	66045	12.5	645573
3.75	96933	18.75	962324
5	129266	25	1240731
6.25	161730	31.25	1559153
7.5	189991	37.5	1837172

D) Accuracy

To test the proposed method's precision, various concentrations of Ivabradine and Metoprolol were taken and analyzed using the proposed method. The accuracy of Ivabradine and Metoprolol was tested by spiking the drug to the pre-analyzed drug solutions at three distinct levels of the test concentration (i.e., 50%, 100%, and 150%) and three times at each level of the test concentration. The average percent Recovery and the average percent RSD were computed for comparison. It was determined that the percentage of recovery was between 98.0 percent and 102.0 percent.

TABLE 3: Recovery study

Drug name	Recovery level	Recovery (%) n=3
Ivabradine	50%	99.5
	100%	99.8
	150%	99.8
Metoprolol	50%	99.72
	100%	99.62
	150%	99.46

E) Precision

Freshly made solutions of Ivabradine and Metoprolol (5 and 25 g/mL, respectively) were used to assess the method's applicability. The improved chromatographic system was injected with 2 L of solution.

TABLE 4: Intermediate precision data

Variation		% RSD for assay of Ivabradine	% RSD for assay of Metoprolol
Different system	Schimadzu UPLC 1	0.8	0.26
	Schimadzu UPLC 2	0.7	0.24
Different column	Batch 1	0.4	0.75
	Batch 2	0.4	0.72
Different analyst	Analyst 1	0.5	0.61
	Analyst 2	0.49	0.62

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The inter-day precisions were determined by analyzing a mixed solution containing 50 µg/mL of Ivabradine and 250 µg/mL of Metoprolol. An intermediate precision was determined on two consecutive day's different instrument.

F) Limit of Detection and Limit of Quantification

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

TABLE 5: LOD and LOQ values of the method

S. No	Parameter	Ivabradine	Metoprolol
1	LOD	0.06	0.28
2	LOQ	0.19	0.85

G) Robustness

It was possible to test robustness by altering the chromatographic conditions deliberately while also measuring the system appropriateness characteristics for both medications. The flow rate, column oven temperature, and mobile phase composition were all tested under these circumstances. On a combined standard solution of Ivabradine and Metoprolol, the investigation was done. In these settings, the findings remained unchanged by minor changes (Table 6).

TABLE 6: Results from study of robustness

Condition	Retention time		% Assay	
	Ivabradine	Metoprolol	Ivabradine	Metoprolol
Water: Methanol (60:40% v/v)	1.191	1.580	99.59	101.41
Water: Methanol (80:20% v/v)	1.427	2.050	99.80	99.20
0.4 mL/min	1.429	1.945	100.50	99.58
0.6 mL/min	1.029	1.402	99.89	98.9
28°C	1.131	1.847	99.25	100.23
32°C	1.100	1.452	99.51	99.79

IV. Results

Different parameters were studied in order to establish the current analytical approach. As a result of its superb peak forms, the Hibar C18 100 X 2.1mm 2m column was chosen in the investigation. For both medications, the optimum max was determined to be 260 nm since the peak purity was satisfactory. To get the best peak area, we used a 2 L injection volume. The flow rate was set to 0.5 mL/min in order to achieve the desired retention periods in the samples. It was discovered that a 50:50 v/v combination of water and acetonitrile was the best solution for the intended investigation since it effectively resolved the medicines. The investigation of Ivabradine and Metoprolol showed maxima at 1.197 and 1.628 0.02 min, respectively, hence 3-min run duration was chosen. The recovery rate ranged from 98.0 to 102.0 percent. Analysis of Ivabradine and Metoprolol determined to be linear in the concentration range of 1.25-7.5 µg/mL and 6.25-37.5 µg/mL respectively. Both the robustness and the ruggedness tests were passed using the analytical approach. The relative standard deviation was less than two in both situations.

V. Discussion

There are several techniques for determining Ivabradine and Metoprolol independently, and few methods in combination with other drugs. For measurement of Ivabradine and Metoprolol in combination dosage forms, numerous HPLC techniques were published. There is also a UPLC method for this combination with linearity range of 5-30µg/ml and 25-150µg/ml for Ivabradine and Metoprolol respectively. (8-19)

VI. Conclusion

The current UPLC technique developed is much more sensitive in terms of Linearity (1.25-7.5 & 6.25-37.5µg/ml respectively for Ivabradine and Metoprolol) and Accuracy also being Eco friendly (Water-Methanol mobile phase) than the already available UPLC method. The approach was validated in accordance with ICH guidelines.

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