QUANTIFICATION OF IVABRADINE AND METOPROLOL BY STRONG CATION EXCHANGE CHROMATOGRAPHY.

D. K. Kadam*[1], S. Sharma[1], S. K. Paliwal[1]

ABSTRACT

It is the first strong cation exchange chromatography estimation of Ivabradine and Metoprolol. Several studies have examined Ranolazine and Dronedarone together. No one has revealed their UV sensitivity. The reported methods were further challenging because they required sophisticated elution to estimate both medications simultaneously. Their analysis time was long, increasing solvent consumption and analysis time. As a result, the simultaneous estimation of Ivabradine and Metoprolol carried out at 223 nm with resolution (R) and capacity factor (k) which were both within acceptable ranges. This whole process took around 10 minutes. Thus, UltraSil-MCX column eluent compositions, buffers, and elution modes were explored to increase specificity and selectivity. Isocratic elution with 10mM ammonium formate (AF)-methanol (20:80 *v/v*) for 10 minutes with considering isocratic elution at 1.2 ml/minute flow rate. The proposed method is validated as per the guidelines.

Keywords: Ivabradine, Metoprolol, Ion exchange chromatography, Forced degradation study

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INTRODUCTION

Ivabradine is chemically 3-[3-({[(7S)-3,4-dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl]methyl}(methyl) amino) propyl]-7,8-dimethoxy-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one used as antihypertensive agent. It acts by inhibiting hyperpolarization-activated cyclic nucleotide-gated (HCN) channel.

Metoprolol is chemically (2*R*,3*R*)-2,3-dihydroxybutanedioic acid;1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino)propan-2-ol used as antihypertensive agent. It is a selective β_1 receptor blocker.

Ivabradine and Metoprolol Succinate combination is used in the treatment of Angina Pectoris. They work together by reducing the heart rate and making the heart more efficient at pumping blood throughout the body. This combination decreases angina symptoms and improves Quality of Life (QoL) in patients with stable angina and coronary artery disease (CAD).[1]

Some methods are reported for Ivabradine and Metoprolol, alone or in combination with other drug molecules.[2] Several research papers have been published on the simultaneous analysis of Ivabradine and Metoprolol using C18 column.[3, 4, 5-10] However, no one has attempted the simultaneous analysis of both drugs using the ion exchange chromatography- specifically, Cation Exchange Chromatography (SCX) method for the separation of this combination. One research paper was observed for the separation of the drug combination of Benidepine and Metoprolol using Cation Exchange Chromatography (SCX) method.[11] Importantly, the significance of this separation technique is that it improves separation efficiency, peak selectivity and resolutions

The proposed HPLC Method for simultaneous quantification of Ivabradine and Metoprolol was validated as per the ICH guidelines and therefore including system suitability studies, other variables such as linearity, accuracy, precision (intra/intermediate) robustness and specificity studies were tested, evaluated and displayed in **Table 1.**

Table No. 1: System suitability of Ivabradine and Metoprolol

System suitability parameters	Ivabradine	Metoprolol	Acceptable Values
Theoretical plates (*N*)	443	536	> **2000**
Capacity Factor (*K'*)	3.41	11.18	> **1.5 - <10**
Resolution (*R*)	---	1.91	**≥ 2**
Selectivity/Separation factor (α)	3.23	2.38	> ***k'***
Asymmetry/Tailing factor (*T*)	1.62	1.53	> **2**
Retention time (t_R)	1.77 min.	4.90 min.	> ***k'***
Wavelength of Detection (nm)	223 nm	223 nm	> **200 nm**
Repeatability (%RSD)	1.65	0.88	< **2**
Intra-Day Precision (%RSD)	0.93-1.71	0.41-1.20	< **2**
Inter-Day Precision (%RSD)	0.89-2.28	0.16-0.34	< **2**
Linearity range	32.5 – 500 µg/ml	16.25 – 250 µg/ml	**NA**
Regression equation	y =17540x + 207352	y =22397x + 1736.2	**NA**
SE of intercept (S_e)	64316.90302	41702.67714	**NA**
SD of intercept (S_a)	143816.9673	93250.02093	**NA**
Correlation Coefficient (r^2)	0.999	0.9998	**NA**
LOQ[a] ($\mu g.mL^{-1}$)	12.10 µg/ml	18.61 µg/ml	**NA**
LOD[a] ($\mu g.mL^{-1}$)	36.66 µg/ml	6.14 µg/ml	**NA**

EXPERIMENTAL

Materials:

Generous gifts of standards; Ivabradine and Metoprolol were received from UltraChrom Innovatives Pvt. Ltd, India. The Metolar I 25 Tablet**,** containing 5 mg of Ivabradine and 25 mg of Metoprolol, manufactured by Cipla Ltd, were purchased from pharmacy. All HPLC grade chemicals and solvents were purchased from Merck (Mumbai, India). The HPLC columns included ProSwift® SCX-1S (50 × 4.6 mm ID; monolith); SiliaBond® Propylsulfonic Acid (SCX-2); Phenomenex-Luna® SCX-3 (100 × 2.1 mm *i.d.* 5µ) purchased from UltraChrom Innovatives Pvt. Ltd. (Nagpur, India). HPLC analysis was performed on Shimadzu Class A-10 VP instrument, equipped with UV-Vis detector (SPD-10A VP), binary pumps (LC-10AT VP), system controller (SCL-10A VP) with manual rheodyne injector (20 µl), controlled by LC-solution software. Analytical balance (ME-205, Mettler-Toledo), pH Mettler (FiveEasy-A211, Mettler -Toledo), and sonicator (Labman®, PCI) were used throughout the analysis.

Chromatographic Conditions:

HPLC analysis was performed on Shimadzu HPLC system. Mobile phases A and B were water and

Methanol, respectively. Both contained 15 mM ammonium formate (AF). Ivabradine and Metoprolol were eluted with AF (15 mM): MeOH in ratio 20:80 *v/v* for 10 minutes with considering isocratic elution at 1.2 ml/minute flow rate. All separations were performed at 28°C and recorded at 230 nm wavelength.

Preparation of Analytical Solutions

Standard preparation

Accurately weighed 25 mg of each standard, Ivabradine and Metoprolol were diluted with 25 ml blank eluents separately in 50 ml volumetric flask and sonicated for 20 minutes. Furthermore, the stock solution was filtered through 0.20 µm nylon filters and volume was adjusted to 50 ml with relevant solvents to make 500 ppm. Furthermore, serial dilutions of different concentrations were made by mixing both standards to determine their validation parameters.

Sample preparation

Twenty Metolar I 25 tablets were weighed separately and accurately. They were crushed to fine powder and then weighed accurately equivalent to 5 mg Ivabradine and 25 mg Metoprolol were transferred to a 100-ml beaker. Each powder was then mixed with 25–50 ml Methanol with continuous stirring for 10 minutes, followed by filtering through 0.20 µm nylon membrane filters into a 100-ml volumetric flask and then volume was adjusted with same eluent. Further serial dilutions were made and then developed SCX-HPLC methods were evaluated for Ivabradine and Metoprolol in their pharmaceutical formulation. Furthermore, the analyte concentration was calculated from their corresponding regression equations.

Method Validation Procedures

Precision

Precision results were expressed in relative standard deviation (RSD). In repeatability, standard stock solution of Ivabradine (500 µg/ml) and Metoprolol (250 µg/ml) was injected six times a day and their resultant peak areas and RSD were determined. Similarly, in intraday and intermediate precision (three different days) the triplicate of standard stock solution containing 500, 250, and 125 µg/ml of Ivabradine; and 100, 50, and 25 µg/ml of Metoprolol were injected thrice and their respective RSD were calculated.

Linearity and range

Linearity was determined by using the calibration curve for both Ivabradine and Metoprolol in the range of 31.25–500 µg/ ml (31.25, 62.5, 125, 250, 500 µg/ml). Prior to that, both standards Ivabradine and Metoprolol were independently dissolved in 15 Mm AF-MeOH eluent to make the concentration of 1 mg/ml and then they were mixed and diluted with the same eluent to obtain the serial dilutions. Linearity of peak area against the concentration was calculated to get regression values and correlation coefficient (r^2).

Limit of detection (LOD) and quantification

LOD and limit of quantification (LOQ) were determined by injecting the homologous mixture of Ivabradine and Metoprolol standard solutions in the range of 0.05–1 µg/ml. Furthermore, the LOD and LOQ were calculated using the following formula:

$$LOD = 3.3 \times \frac{\text{Std. Deviation of intercept}}{\text{Slope}}$$

$$LOQ = 10 \times \frac{\text{Std. Deviation of intercept}}{\text{Slope}}$$

Robustness

The robustness studies involved the small variations in selected separation parameters such as changes in flow rate (±0.2 ml/minutes) organic modifier concentration (±0.2 ml), and temperature (±2°C) were tested and evaluated. The flow rate of the eluent was changed from 1 ml/minutes to 0.8 and 1.2 ml/minutes; the concentration of organic modifier was changed from 20% to 18% and 22% and the temperature was changed from 32°C to 30 °C and 34 °C for both Ivabradine and Metoprolol. Furthermore, the results derived were evaluated for any changes in capacity factor (k'), resolution (R), theoretical plates (N), and tailing factor (T).

Accuracy

The accuracy was determined by mixing the fixed concentration of standards, Ivabradine (2.5 µg/ml) and Metoprolol (250 µg/ ml) with varying concentrations of Metolar I 25 tablets as 2 µg, 2.5 µg, and 3 µg to make the 80%, 100%, and 120%, respectively. The analysis was performed in a triplicate with data calculated to determine the percentage (%) drug recovery, mean ± SD, and percentage (%) RSD.

Degradation studies

Acid, Alkali, oxidation and thermal degradation studies

Forced degradation studies of Ivabradine and Metoprolol were performed as per the International Conference on Harmonization (ICH) guideline[12]. 8 ml of freshly prepared homologous mixture of stock solution, containing Ivabradine (500 µg/ml) and Metoprolol (250 µg/ml), prepared in H_2O–Methanol eluents was equally distributed into 4 different 25 ml volumetric flasks and further

diluted with equal volume of H_2O, 0.1 N HCl, 0.1N NaOH, and 3% H_2O_2 to get final concentration of 250 µg/ml and 50 µg/ml of Ivabradine and Metoprolol, respectively. Sample prepared in 3% H_2O_2 was kept at room temperature for 6 hours whereas the acid-base and neutral hydrolyzed samples were kept at 60°C for 6 hours. Furthermore, all samples were sonicated, filtered through 0.20 µm nylon filters and then twenty µL of each sample was analyzed by HPLC using specified chromatographic method mentioned in chromatographic condition.

RESULT AND DISCUSSIONS

System Suitability Study:

The proposed HPLC method for simultaneous quantification of Ivabradine and Metoprolol was validated as per the ICH guidelines and therefore including system suitability studies, other variables such as linearity, accuracy, precision (intra/intermediate) robustness and specificity studies were tested, evaluated and displayed in **Table 1**. As demonstrated, the system suitability parameters of the proposed method represent a high degree of reproducibility for simultaneous quantification of Ivabradine and Metoprolol. For Ivabradine, developed method expressed average retention time (t_R) of 1.33 minutes with mean k' of 1.52

Whereas the t_R and k' for Metoprolol were 2.44 minutes and 3.34, respectively (**Table 1**). The tailing factor (*T*) values <2 signified no specific tailing in both analytes. Symmetric peaks represent an ideal Gaussian peak; where for both compounds, the symmetric and asymmetric factors were of equal magnitude. The separation factor (α) and resolution (R_s) for both Ivabradine and Metoprolol were found significantly higher than the minimum requirement as per the ICH guidelines (**Table 1**).

After selecting SCX chromatography for simultaneous analysis of selected drugs, several trials including the eluent compositions, buffers selection and elution mode were tested and evaluated (**Figure 1**). Finally, the separation carried out in UltraSil-MCX column with isocratic elution, consisting 15mM ammonium formate (AF)- methanol (20:80 *v/v*) explicit the best results. UV detection was specifically carried out at 230 nm for both Ivabradine and Metoprolol as both compounds exhibit optimum absorption at above selected wavelengths. The flow rate was adjusted to 1.2 mL/min to achieve better resolution, and better peak symmetry.

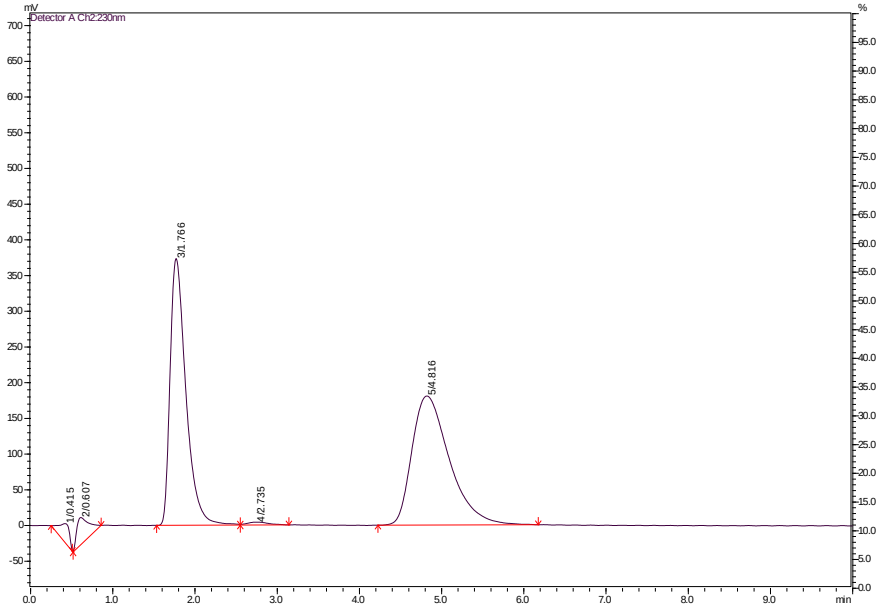

Figure 1: Metoprolol and Ivabradine with 15mM AF-MeOH (20:80 *v/v*)

Method validation

The method was validated according to ICH guidelines.

Repeatability

Implementing the procedure mentioned under experimental section, the homologous mixture of both Ivabradine (250 µg/ml) and Metoprolol of concentrations (500 µg/ml), were tested for six injections within the same day. The % RSD was calculated and found they were 1.65 and 0.88 for Ivabradine and Metoprolol, respectively which is less than 2% as shown in (**Table 2**).

Table 2: Repeatability data of Ivabradine and Metoprolol

Sr. No.	Ivabradine: 223 nm	Metoprolol: 223 nm
	Peak Area: Conc. 250 ppm	Peak Area: Conc. 500 ppm
1	105147	5602798
2	107934	5618176
3	107968	5647769
4	106456	5653480
5	106521	5706691
6	103373	5730306
Mean	**106233.1667**	**5659870**
STD. DEV.	**1754.72**	**49671.53**
RSD (%)	**1.65**	**0.88**

Intraday precision:

Implementing the procedure mentioned under experimental section, the homologous mixture of both Ivabradine (500 ppm) and Metoprolol (250 ppm) of three replicates were tested and evaluated within the same day (intra-day precision). The % RSD was calculated and found it is less than 2%; shown in (**Table 3 and 4**).

Table 3: Intraday Precision data of Ivabradine

Sr. No.	Concentration (ppm)	Area	Mean ± SD	%RSD
1	500 ppm	105147	1618.97	1.51
	500 ppm	107934		
	500 ppm	107968		
2	500 ppm	106456	1799.02	1.71
	500 ppm	106521		
	500 ppm	103373		
3	500 ppm	106644	985.03	0.93
	500 ppm	105352		
	500 ppm	104710		
Range of % RSD				**0.93-1.71**

Table 4: Intraday Precision data of Metoprolol

Sr. No.	Concentration (ppm)	Area	Mean ± SD	%RSD
1	250 ppm	5602798	22856.87123	0.41
	250 ppm	5618176		
	250 ppm	5647769		
2	250 ppm	5653480	39351.64762	0.69
	250 ppm	5706691		
	250 ppm	5730306		
3	250 ppm	5702585	67725.60305	1.20
	250 ppm	5579642		
	250 ppm	5690348		
Range of % RSD				**0.41-1.20**

Interday (Intermediate) Precision:

Implementing the procedure mentioned under experimental section**,** the homologous mixture of both Metoprolol and Ivabradine of three replicates of three different concentrations; 250 ppm, 500 ppm, respectively were tested and evaluated in three successive days (interday/intermediate precision). The %RSD was calculated and found it is less than 2%; as displayed in (**Table 5 and 6**).

Table 5: Interday (Intermediate) Precision Data of Ivabradine

Sr. No.	Concentration (ppm)	Area	Mean ± SD	%RSD
DAY 1	500 ppm	105147	1618.979411	1.51
	500 ppm	107934		
	500 ppm	107968		
DAY 2	500 ppm	105001	2336.16873	2.28
	500 ppm	100390		
	500 ppm	102042		
DAY 3	500 ppm	102265	898.8796916	0.89
	500 ppm	100682		
	500 ppm	100517		
Range of % RSD				**0.89-2.28**

Table 6: Interday (Intermediate) Precision Data of Metoprolol

Sr. No.	Concentration (ppm)	Area	Mean ± SD	% RSD
DAY 1	500 ppm	5602798	22856.87123	0.41
	500 ppm	5618176		
	500 ppm	5647769		
DAY 2	500 ppm	5693901	19551.84355	0.34
	500 ppm	5711338		
	500 ppm	5732931		
DAY 3	500 ppm	5781638	9085.039956	0.16
	500 ppm	5794861		
	500 ppm	5799042		
Range of % RSD				**0.16-0.34**

Linearity and range

The linearity of any HPLC method, is its ability to explicit the results that should be proportional to the concentration of studied analytes within a selected range. Therefore, over the range of 32.5–500 µg/ml for Ivabradine and 16.25–250 µg/ml for Metoprolol, exceptionally high linearity was observed between the concentration against peak area with linear regression observed for Ivabradine and Metoprolol were; *y=17540x+207352*; and *y=22397x–1736.2*, respectively (**Figure 2 and 3**). Moreover, the regression coefficients (r^2) were almost 0.999 for both samples; which itself designated a high degree of linearity (**Table 7 and 8**).

Table 7: Linearity Data of Ivabradine

Sr. No.	Concentration (µg/mL)	Area	Average (Mean)
1	500 ppm	8921124	8921124
2	250 ppm	4680257	4680257
3	125 ppm	2489752	2489752
4	62.5 ppm	1261647	1261647
5	31.25 ppm	675820	675820
6	Regression Equation		*y = 17540x + 207352*
7	Correlation coefficient (R^2)		0.9994
8	Std. Error of intercept		64316.90302
9	Std. Dev. of intercept		143816.9673
10	LOQ		36.66 µg/ml
11	LOD		12.10 µg/ml

Table 8: Linearity Data of Metoprolol

Sr. No.	Concentration (µg/mL)	Area	Average (Mean)
1	500 ppm	11234276	11234276
2	250 ppm	5516389	5516389
3	125 ppm	2835205	2835205
4	62.5 ppm	1379943	1379943
5	31.25 ppm	739796	739796
6	Regression Equation		*y = 22397x + 1736.2*
7	Correlation coefficient (R^2)		0.9998
8	Std. Error of intercept		41702.67714
9	Std. Dev. of intercept		93250.02093
10	LOD		18.61 µg/ml
11	LOQ		6.14 µg/ml

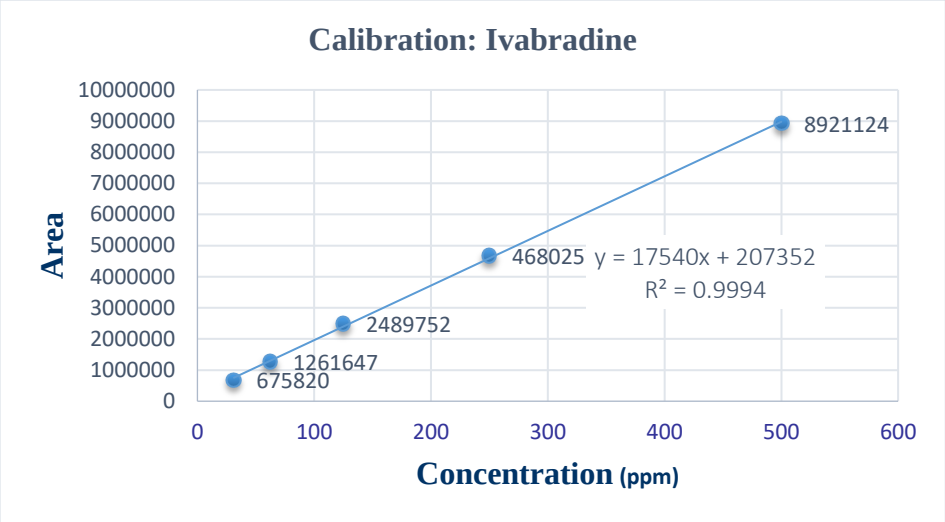

Figure 2: Calibration Curve of Ivabradine

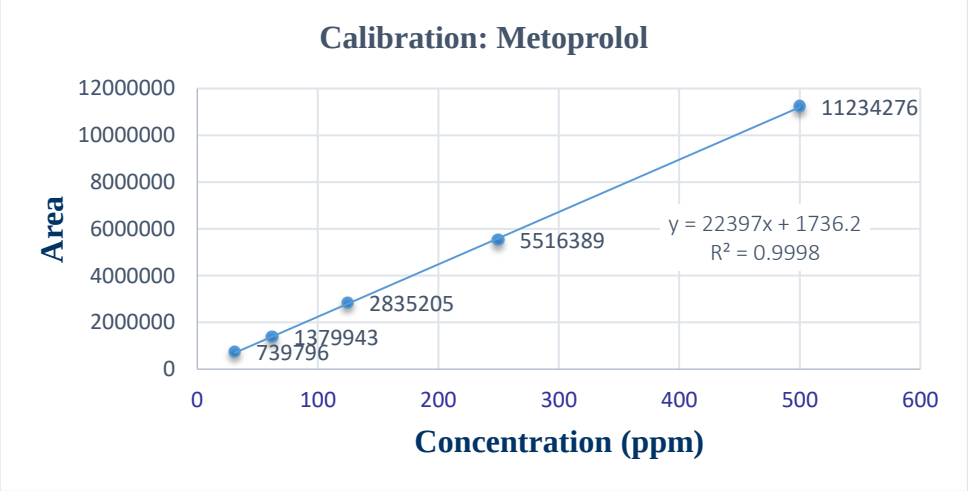

Figure 3: Calibration Curve of Metoprolol

Limit of detection (LOD) and quantification (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were estimated based on the standard deviation of the response and the slope of the regression equation. As observed, The LOD and LOQ of Ivabradine were 12.10 and 36.66 µg/ml whereas for Metoprolol they were 6.14 and 18.61 µg/ml, respectively. It signified the higher detection ability of the method for the lowest possible concentration of simultaneous investigation of selected drugs from the combination.

Robustness for the Chromatographic Method

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor). Robustness was studied for Metoprolol and Ivabradine, results obtained was displayed in **Table 9 and 10**. As resulted, the flow rate of the mobile phase was changed from 1.2 mL/min to 1.4 mL/min and 1.0 mL/min; results shown in **Table 9 and 10**. Similarly, the effect of deliberate changes in organic modifier (methanol) composition was evaluated. In this study, the percentage composition of methanol was altered by ±2% in the previous set of gradient to evaluate the effects on the separation behaviour of Ivabradine and Metoprolol results shown in **Table 11 and 12**. Finally, the temperature was changed by ±2˚C and the results were reported in **Table 13 and 14**.

From all above studies, after making deliberated changes in flow rate (± 0.2 mL/min), organic modifier concentration as methanol (±2%) and temperature (±2˚C) have not made any significant changes in resolution, capacity factor and tailing factor. Nonetheless, it seems minute changes in robustness studies makes significant changes in theoretical plate counts. Robustness studies for Ivabradine and Metoprolol displayed in **Table 11 and 12**; and **Figure 4 to 10.**

Table No. 9: Retention Parameters of Robustness Studies at Flow Rate 1.4 ml/min

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.358	185663	1.8513	47.761	--	0	0.792	0
2	0.527	328881	3.2793	77.093	0.752	0.469	1.898	0
Ivabradine	1.522	4432037	44.1925	368.076	3.573	3.248	1.614	6.92
Metoprolol	4.217	5082345	50.6769	499.658	5.027	10.765	1.462	3.315

Table No. 10: Retention Parameters of Robustness Studies at Flow Rate 1.0 ml/min

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.492	208804	1.4801	88.847	--	0	0.95	0
2	0.717	687376	4.8724	66.068	0.804	0.459	2.319	0
Ivabradine	2.105	6081345	43.1072	459.76	3.72	3.28	1.611	7.145
Metoprolol	5.783	7129975	50.5403	608.046	5.529	10.761	1.491	3.281

Table No. 11: Retention Parameters of Robustness Studies with 15mM AF-MeOH (82:18 *v/v*)

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.432	336093	2.7961	49.425	--	0	0.734	0
2	0.617	670720	5.5801	80.432	0.711	0.429	2.091	0
Ivabradine	1.782	4989712	41.512	405.214	3.7	3.123	1.59	7.284
Metoprolol	5.183	6023400	50.1118	568.593	5.56	10.994	1.441	3.52

Table No. 12: Retention Parameters of Robustness Studies with 15mM AF-MeOH (78:22 *v/v*)

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.416	106698	0.947	103.08	--	0	1.042	0
2	0.608	370892	3.2919	66.536	0.832	0.462	2.202	0
Ivabradine	1.817	4792091	42.5322	373.208	3.585	3.369	1.595	7.287
Metoprolol	5.061	5997290	53.2289	504.207	5.078	11.171	1.522	3.315

Table No. 13: Retention Parameters of Robustness Studies at temperature 30˚C

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.407	174738	1.16	84.162	--	0	1.047	0
2	0.61	536401	3.561	61.805	0.832	0.498	2.154	0
Ivabradine	1.763	8151614	54.1162	433.559	3.555	3.333	1.599	6.687
Metoprolol	4.799	6200413	41.1627	547.197	5.238	10.795	1.463	3.239

Table No. 14: Retention Parameters of Robustness Studies- at temperature 34˚C

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.407	174738	1.16	84.162	--	0	1.047	0
2	0.61	536401	3.561	61.805	0.832	0.498	2.154	0
Ivabradine	1.763	8151614	54.1162	433.559	3.555	3.333	1.599	6.687
Metoprolol	4.799	6200413	41.1627	547.197	5.238	10.795	1.463	3.239

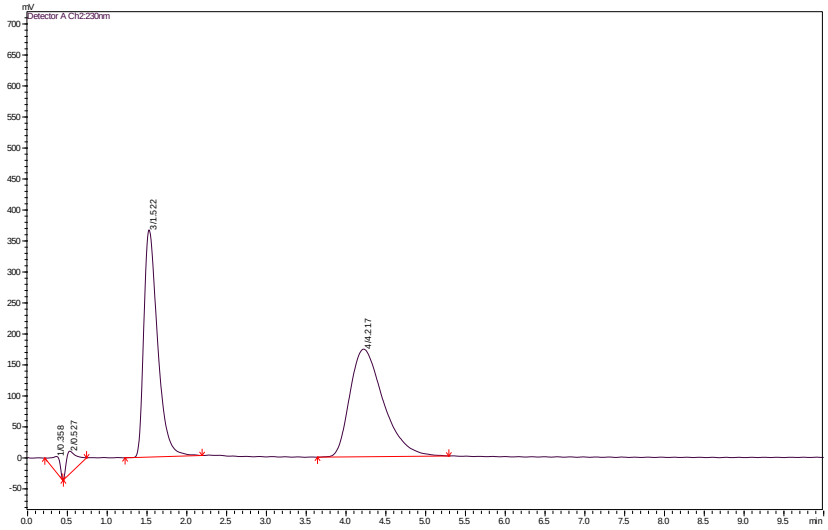

Figure 4: Robustness Studies of Ivabradine and Metoprolol at Flow Rate 1.4 ml/min

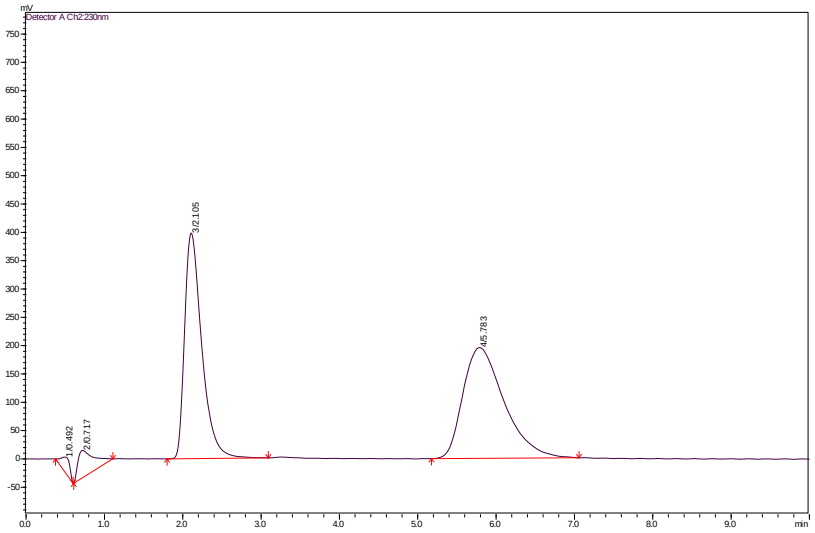

Figure 5: Robustness Studies of Ivabradine and Metoprolol at Flow Rate 1.0 ml/min

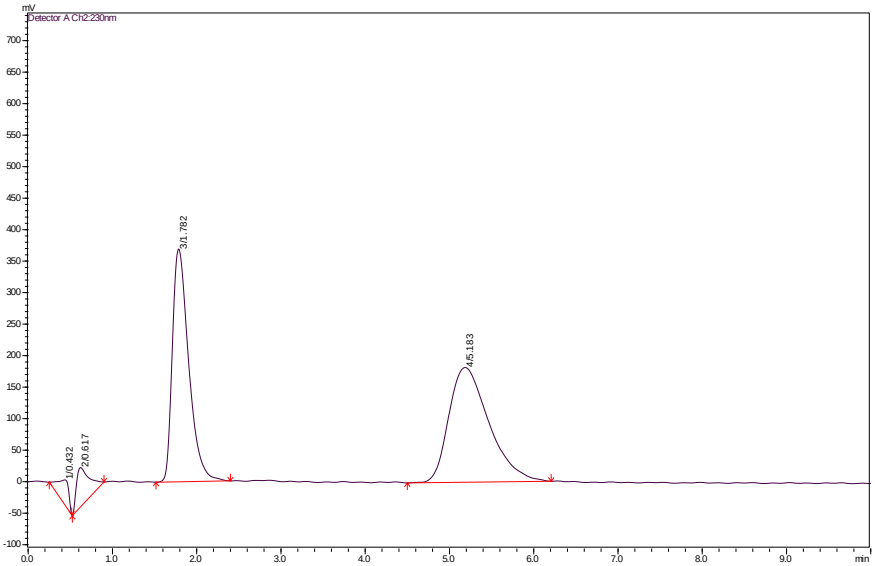

Figure 6: Robustness Studies of Ivabradine and Metoprolol with 15mM AF-MeOH (82:18 *v/v*)

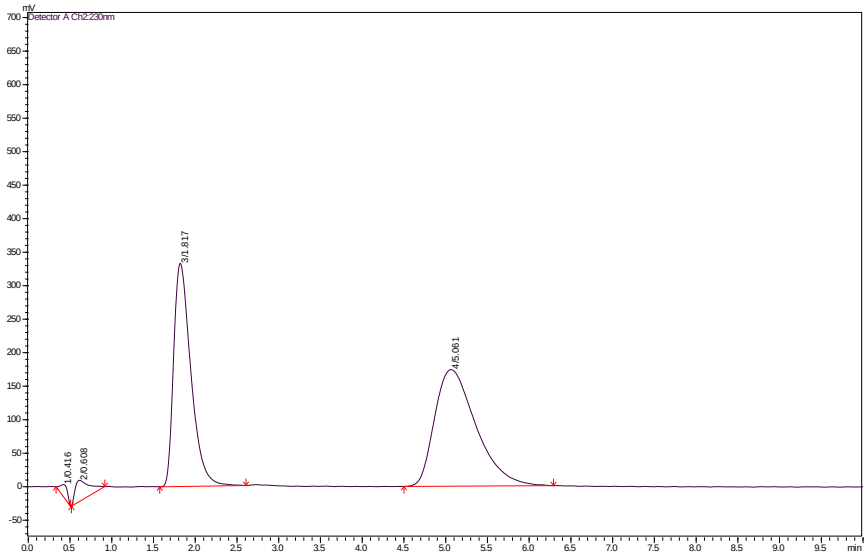

Figure 7: Robustness Studies of Ivabradine and Metoprolol with 15mM AF-MeOH (78:22 *v/v*)

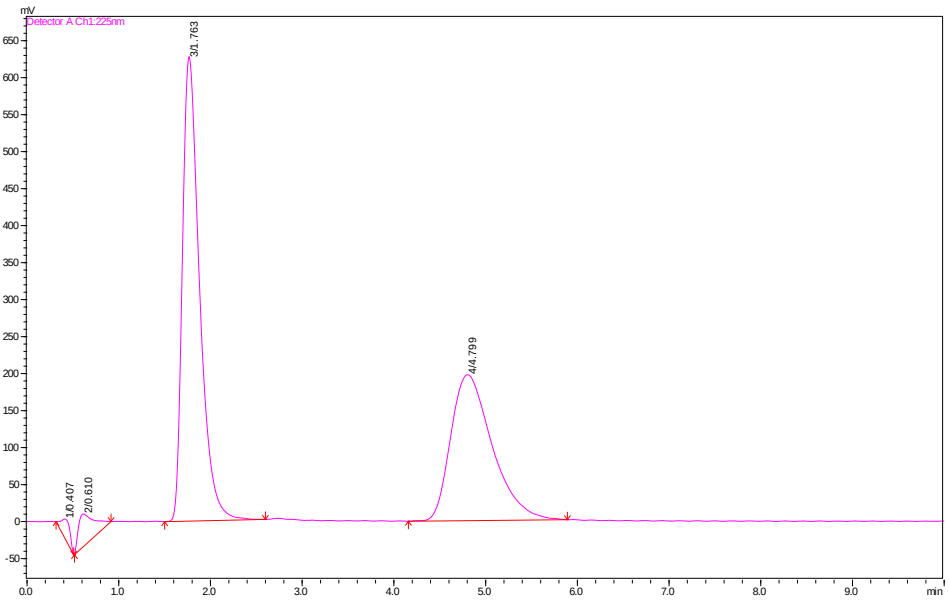

Figure 8: Robustness Studies of Ivabradine and Metoprolol at temperature 30˚C

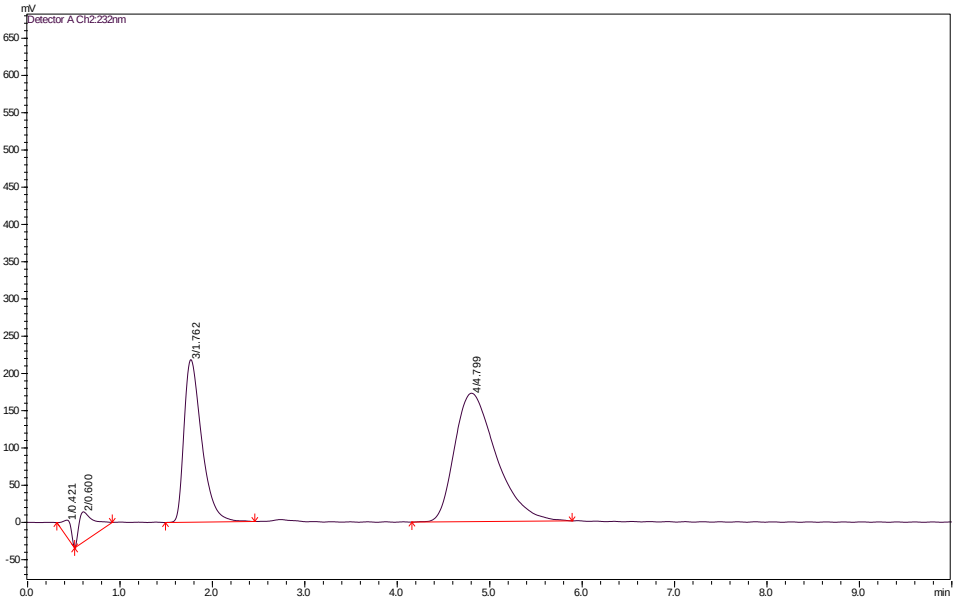

Figure 9: Robustness Studies of Ivabradine and Metoprolol at temperature 34˚C

Accuracy Studies of Ivabradine and Metoprolol

Accuracy of the results was calculated by % recovery of 3 different concentrations in combination of each drug. The results including the mean of the recovery and standard deviation are shown in **Table 15 and 16**. Percentage recoveries of three different concentrations (injected thrice) to determine the Ivabradine and Metoprolol were calculated to demonstrate the accuracy in RSD% for the selected pharmaceutical combination reported in **Table 15 and 16**. Moreover, alternatively it can also calculated by applying the calibration curve, the Y-intercept and the slope of the graph to determine the % recovery, attributed to the proposed method for the simultaneous quantification. As resulted, the % RSD of Ivabradine was in the range of 0.01, 1.40, and 0.03 and for Metoprolol it was 0.43, 0.13, and 0.43 which are within the ICH and USP acceptance limit of ±2% (**Table 1**). Overall, the method displayed good accuracy from the obtained recovery data.

Table No. 15: Drug Recovery Study of Ivabradine

Conc. (%)	No.	Std. (mg)	Drug (mg)	Rec. (mg)	% recovery	Area = (500 ppm)	Mean Rec. (%)	STD. Dev.	% RSD
80%	1	100	80	180.1	100.06	8577566	100	0.01	0.01
	2	100	80	180.12	100.07	8578518			
	3	100	80	180.11	100.06	8578042			
100%	1	100	100	195.25	97.63	9299110	99	1.39	1.40
	2	100	100	200.05	100.03	9527718			
	3	100	100	200.07	100.04	9528671			
120%	1	100	120	220.17	100.08	10485967	100	0.03	0.03
	2	100	120	220.24	100.11	10489301			
	3	100	120	220.13	100.06	10484062			

Table No. 16: Drug Recovery Study of Metoprolol

Conc. (%)	No.	Std. (mg)	Drug (mg)	Rec. (mg)	% recovery	Area = (500 ppm)	Mean Rec. (%)	STD. Dev.	% RSD
80%	1	500	400	90.81	100.90	21697321	100.74	0.43	0.43
	2	500	400	90.23	100.26	21558741			
	3	500	400	90.97	101.08	21735549			
100%	1	500	500	101.12	101.12	24160699	101.02	0.13	0.13
	2	500	500	101.06	101.06	24146363			
	3	500	500	100.87	100.87	24100966			
120%	1	500	600	109.27	99.34	26107986	99.98	0.43	0.43
	2	500	600	110.17	100.15	26323024			
	3	500	600	109.98	99.98	26277627			

Forced degradation studies

The forced degradation studies of Ivabradine and Metoprolol using SCX chromatography revealed possible degradation under the influence of acid-base strength, peroxide and thermal environment. As observed, the compounds were resistant to the 0.1N HCl treatment since no degraded products were appeared in chromatogram (**Figure 11**). Similarly, the treatment under 3% H_2O_2 produced oxidative stress, thermal stress condition at 50˚C and the treatment under 0.1N have not made any significant changes in t_R values of both selected drugs since no any fragments of degradants were appeared in **Figure 10 to 13** and **Table 17 to 22.**

Table No. 17: Force Degradation Studies of Ivabradine

Conditions	No. of degradants (fragments)	% degradation
Acid (0.1N/M HCl) + 60˚C + 12 Hrs.	0 Fragment	0 %
Base (0.1N/M NaOH) + 60˚C + 12 Hrs.	0 Fragments	0 %
Thermal (60˚C) + 12 Hrs.	0 Fragment	0 %
Oxidation (3-6% H_2O_2) + Room Temp.	0 Fragments	0%
Sunlight exposed + 3 days	Not Performed	----

Table No. 18: Force Degradation Studies of Metoprolol

Conditions	No. of degradants (fragments)	% degradation
Acid (0.1N/M HCl) + 60˚C + 12 Hrs.	0 Fragments	0%
Base (0.1N/M NaOH) + 60˚C + 12 Hrs.	0 Fragments	0%
Thermal (60˚C) + 12 Hrs.	0 Fragments	0%
Oxidation (3-6% H_2O_2) + Room Temp.	0 Fragments	0%
Sunlight exposed + 3 days	Not Performed	----

Table No. 19: 0.1N HCl Induced Stress Effect on Ivabradine and Metoprolol

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.322	624418	4.6682	25.366	--	0	2.106	0
Ivabradine	0.87	6752341	50.4809	180.542	2.132	1.705	1.777	0
Metoprolol	1.825	5999260	44.8509	368.158	2.987	4.675	1.575	2.742

Table No. 20: 0.1N NaOH Induced Stress Effect

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.446	560667	4.5693	77.043	--	0	0.811	0
Ivabradine	0.858	6094954	49.6725	171.094	1.772	0.926	1.713	0
Metoprolol	1.698	5614664	45.7582	373.285	2.736	2.81	1.598	3.036

Table No. 21: 3% H_2O_2 Induced Stress Effect

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.242	1447	0.0067	29.365	--	0	1.859	0
Ivabradine	0.899	10442633	48.1352	137.111	2.708	2.72	1.987	0
Metoprolol	1.915	11250282	51.8581	308.746	2.736	6.925	1.681	2.546

Table No. 22: Thermal (50˚C) Induced Stress Effect

Peak #	Retention Time	Area	Area %	Theoretical Plate#	Resolution	k'	Tailing Factor	Separation
1	0.43	207568	1.6273	134.857	--	0	1.095	0
Ivabradine	0.864	6713135	52.6283	165.336	2.082	1.009	1.954	0
Metoprolol	1.849	5835059	45.7445	311.004	2.864	3.301	1.644	3.271

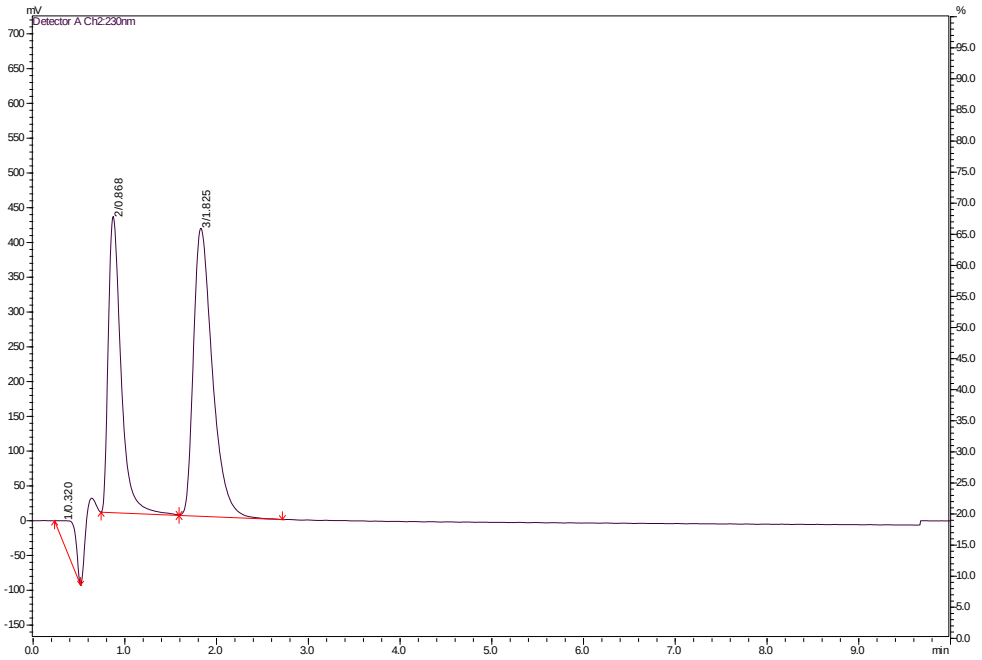

Figure 10: 0.1N HCl Induced Stress Effect on Ivabradine and Metoprolol

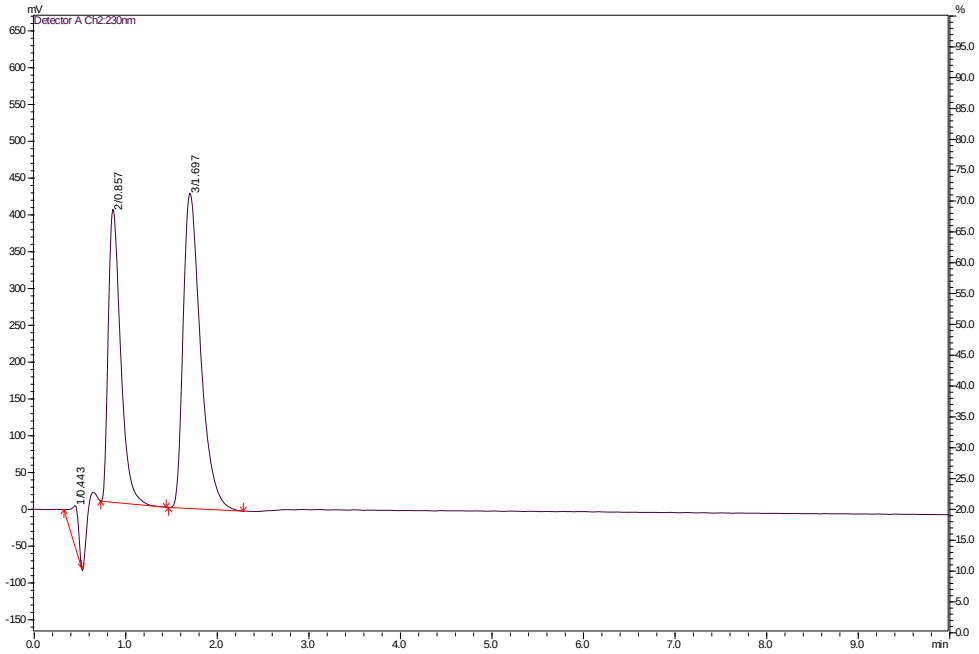

Figure 11: 0.1N NaOH Induced Stress Effect

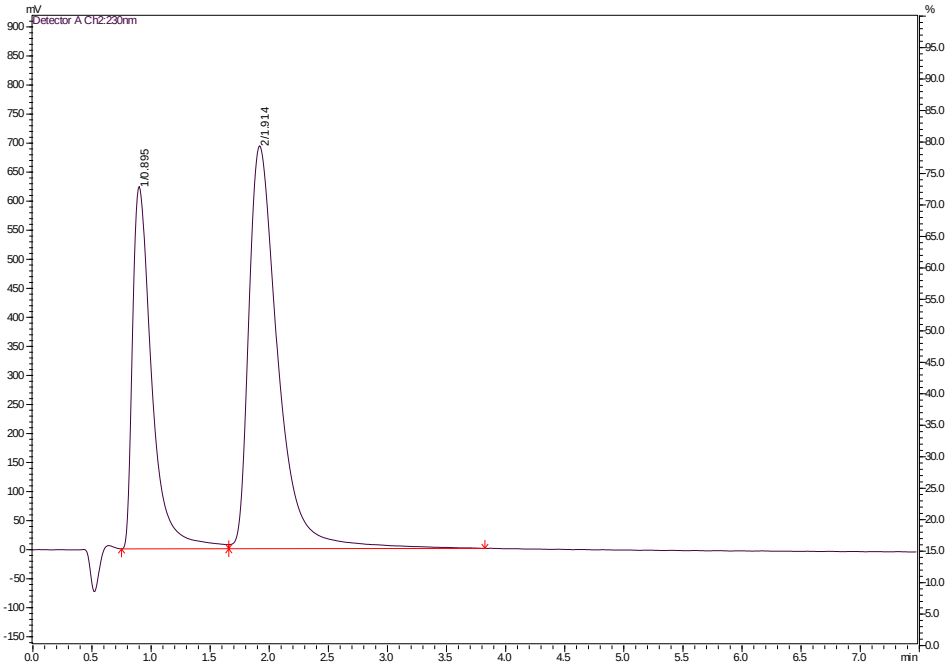

Figure 12: 3% H_2O_2 Induced Stress Effect

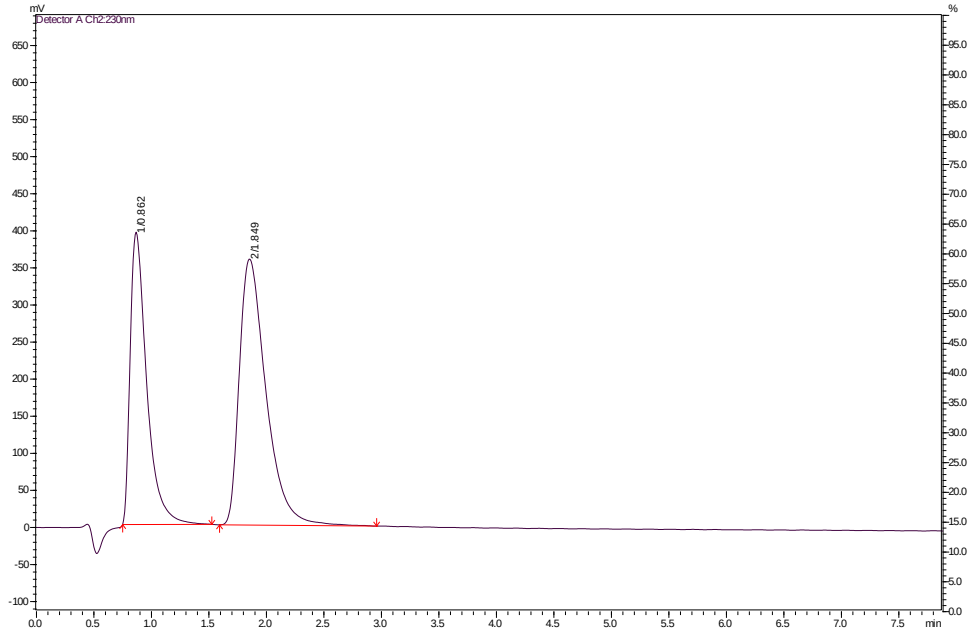

Figure 13: Thermal (50˚C) Induced Stress Effect

CONCLUSION

The suggested method for the estimation of Ivabradine and Metoprolol using Ion Exchange Chromatography was assessed for linearity, precision, accuracy and system suitability; it was found useful for the estimation of these drugs. With a correlation coefficient correlation value of 0.999, it was found accurate and linear across the concentration range examined (31.25–500 µg/ml) at 223nm with resolution (R) and capacity factor (k) in acceptable ranges. It is clear from the results obtained that the suggested method may be used to determine Ivabradine and Metoprolol with high sensitivity without causing any interference. As a result, the proposed method is selective and useful for the quantification of Ivabradine and Metoprolol.

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CONFLICT OF INTEREST

NIL

AUTHOR CONTRIBUTION

All authors contributed equally for the investigation.

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