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Development and validation of RP-HPLC method for analysis of Ivabradine Hydrochloride in tablet dosage forms

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ABSTRACT

A simple High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of ivabradine hydrochloride has been developed and validated. The chromatographic system consisted of a LC-20 AT pump, SPD-20A UV detector, SIL-20A auto-sampler and CTO-10ASVP column oven. The separation was achieved by C_{18} column (VP-ODS, 150 x 4.6 mm, 5 µm) at 35^0 C temperature with a mobile phase consisting of buffer (pH-7.3), methanol and acetonitrile (55:15:30 v/v) pumped at a flow rate of 1ml/min. Retention time was 7.46 minutes. The calibration curves were linear over the concentration range of 50% to 150% of target concentration (r^2 = 1). The proposed method is accurate with 100.37% recovery and precise (%RSD was less than 2%). The method has been used to test marketed tablets and potency was found within 98.7%-100.14%. This method can be used for the analysis of ivabradine hydrochloride in bulk and tablet dosage forms.

Keywords: Ivabradine tablet, HPLC method, validation, accuracy

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INTRODUCTION

Analysis is an important component in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drug(s) in the bulk, in drug delivery systems, from dissolution studies and in biological samples. If a suitable method is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. Ivabradine hydrochloride is a new bradycardiac agent. Compendial method for the analysis of this drug is not available. So development and validation of a suitable method is essential for the routine estimation of ivabradine hydrochloride in bulk and tablet dosage forms.

Ivabradine hydrochloride is a novel medication used for symptomatic management of stable angina pectoris. The drug acts by reducing the heart rate. Preliminary animal study indicates that ivabradine unlike beta- blocking agents, does not have any vasodilatory effect on inotropic properties [1-4].

Literature survey revealed that a liquid chromatography (LC) method using fluorimetric detection and a LC method using mass spectrometric detection were validated to quantify ivabradine in urine and plasma respectively [5-6]. Method for determination and quantification of ivabradine in bulk drug and pharmaceutical dosage form has also been reported [7]. But they are not free from limitations.

Therefore it is very important to have a specific, selective, reliable and cheap method for determination of ivabradine in bulk drug and pharmaceutical dosage forms. So attempt was taken to develop a rapid reversed-phase high performance liquid chromatographic method for the quality control of ivabradine in pharmaceutical preparations with lower solvent consumption and short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow analysis of a large number of samples in a short period of time. The developed method was validated and found to be linier, accurate and precise.

MATERIALS AND METHODS

Material

Ivabradine hydrochloride was provided by Incepta Pharmaceuticals Ltd. Dhaka, Bangladesh. Acetonitrile and methanol were of HPLC grade and were purchased from Lab Scan, Di-ammonium hydrogen phosphate (Riedel-deHaen), Phosphoric acid (Sigma-Aldrih) were collected from local market. Water was deionised and double distilled. Three commercial brands of tablets containing ivabradine hydrochloride were purchased from local drug shops in Dhaka city after checking their manufacturing license numbers, batch numbers, production and expiry dates.

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Instrumentation

A Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A auto-sampler and CTO-10ASVP column oven were used. Ultraviolet detection was achieved with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analysis data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC.

Chromatographic conditions

The mobile phase, a mixture of buffer (pH-7.3), methanol and acetonitrile (55:15:30 v/v) pumped at a flow rate of 1.0 ml/min through the column (C18; 5µ, 4.6 X 150 mm,) at 35°C temperature. The mobile phase was degassed prior to use under vacuum by filtration through a 0.2µ nylon membrane. Concentrations were measured at 285 nm by UV detector at a sensitivity of 0.0001.

Column	:	VP-ODS, C-18, 150 mm X 4.6 mm.
Wavelength (λ)	:	285 nm.
Column Temperature	:	35°C.
Flow Rate	:	1.0 mL/min.
Injection Volume	:	20µL.
Run Time	:	15 min.
Retention time	:	7.46 min.

Preparation of Mobile Phase

Buffer pH-7.3, methanol and acetonitrile at a volume ratio of 55:15:30 were mixed and filtered through a filter having a nominal pore size not greater than 0.2 µm. Finally the mixture was degassed in an ultrasonic bath. Buffer was prepared by dissolving 2.72 g of Di-ammonium hydrogen phosphate in 550 mL of water. The pH of the solution was adjusted to 7.3 with dilute Phosphoric acid.

Preparation of Standard Solution

40 mg of ivabradine hydrochloride standard was taken in a 50-mL volumetric flask and 30 mL mobile phase was added to dissolve it. Finally volume was made up to the mark with mobile phase. 5.0 mL of this standard solution was diluted to 25 mL with mobile phase and filtered through a filter having a nominal pore size 0.2 µm.

Preparation of Test Sample

20 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to a fine powder with the help of mortar and pestle. Then, the amount of powder containing 40 mg ivabradine hydrochloride was transferred to a volumetric flask, dissolved in mobile phase and shaken for about 10 minutes then filtered through filter paper. The filtered

solution was further diluted in the mobile phase to make the final concentration of working sample equivalent to 100% of target concentration.

Development and validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of ivabradine hydrochloride in tablet dosage forms. The experiment was carried out according to the official specifications of USP–30; ICH- 1996, Global Quality Guidelines-2002[8-10].The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness.

System suitability

The system suitability was assessed by six replicate analysis of ivabradine at a 100% level to verify reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area, tailing factor, theoretical plates (Tangent) of the column.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of ivabradine hydrochloride at different concentrations level (50%- 150%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in duplicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of ivabradine hydrochloride to obtain the calibration curves. The seven concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

32.5mg, 36.1mg, 40.0mg, 44.2mg & 47.8mg of Ivabradine Hydrochloride were taken in five different 50-mL volumetric flasks. 450.0 mg of placebo was added in each volumetric flask. Volume was made to the mark with mobile phase. 5 mL of this solution was diluted to 25 mL with mobile phase. These solutions were injected to determine recovered amount. Recovery was calculated by the following formulation:

% Recovery = (Experimental Concentration ÷ Theoretical Concentration) x 100

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Percent relative standard deviation was calculated by using the following relationship:
%RSD = (Standard Deviation ÷ Mean Concentration) x 100%

Precision

The precision of the method was investigated with respect to repeatability (inter assay precision) and intermediate precision. Repeatability was determined by performing repeated analysis of six samples on the same day, under the same experimental conditions. % RSD was calculated to determine the reparability. Intermediate precision of the method was assessed by carrying out the analysis of standard solutions by two analysts in the same laboratory.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 ml/min, amount of methanol (15% to 17%,) the temperature of the column (32 °C to 35 °C).

Specificity

Specificity was determined by injecting separately blank, placebo, standard and test samples.

Analysis of market products

The proposed method was used to determine the potency of commercially available tablets. Six replicate determinations (n=6) were carried out.

RESULTS AND DISCUSSION

System Suitability

The system suitability tests were carried out to evaluate the reproducibility of the system for the analysis. Table 1 summarized the test results of system suitability study (peak area and tailing factor). All the chromatograms showed the same retention time (7.46 min) from the six consecutive injections of the standard solution which indicates a good system for analysis. % RSD for mean peak area and tailing factor were within limit.

Table-1: Result of System Suitability Test

Replicate	Peak area	Tailing Factor	RSD of peak area		Tailing Factor		Pass/Fail
			Limit	Results	Limit	Results	
1	3274485	0.99	NMT2.0	0.085	0.95 to 1.05	0.99	Passed
2	3277480	0.99					
3	3274656	0.99					
4	3274966	1.00					
5	3278616	1.00					
6	3281488	0.99					

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Linearity and range (LLOQ and ULOQ)

Duplicate injections were made for each concentration level. The actual concentrations of the seven standards against the respective peak areas were computed and the linear regression curve was generated. A linear relationship was determined through calculation of a regression line by the method of least squares. A plot of the data as well as the correlation coefficient, y-intercept and slope of the regression line and acceptance criteria was presented in Table 2 and figure 1. The lower limit of quantitation (LLOQ) is the lowest concentration within the linear range (80.60 µg/mL). The upper limit of quantitation (ULOQ) is the highest concentration within the linear range (241.80 µg/mL).

Table-2: Result of Linearity and Range

% of Nominal value	Conc. of Std (µg/mL)	Peak areas	Average peak areas	Regression coefficient (R^2)		y-intercept	Slope of regression line	Pass/ Fail
				Limit	Result			
50%	80.60	1646181	1652588	NLT 0.995	1.00	1.8571	18.199	**Passed**
		1658995						
60%	96.72	1972335	1979948					
		1987560						
80%	128.96	2620585	2628123					
		2635660						
100%	161.20	3275732	3275372					
		3275012						
120%	193.44	3925883	3924549					
		3923215						
140%	225.68	4576342	4569966					
		4563589						
150%	241.80	4900499	4905368					
		4910236						
Lower limit of quantitation (LLOQ)						80.60 µg/mL		
Upper limit of quantitation (ULOQ)						241.80 µg/mL		

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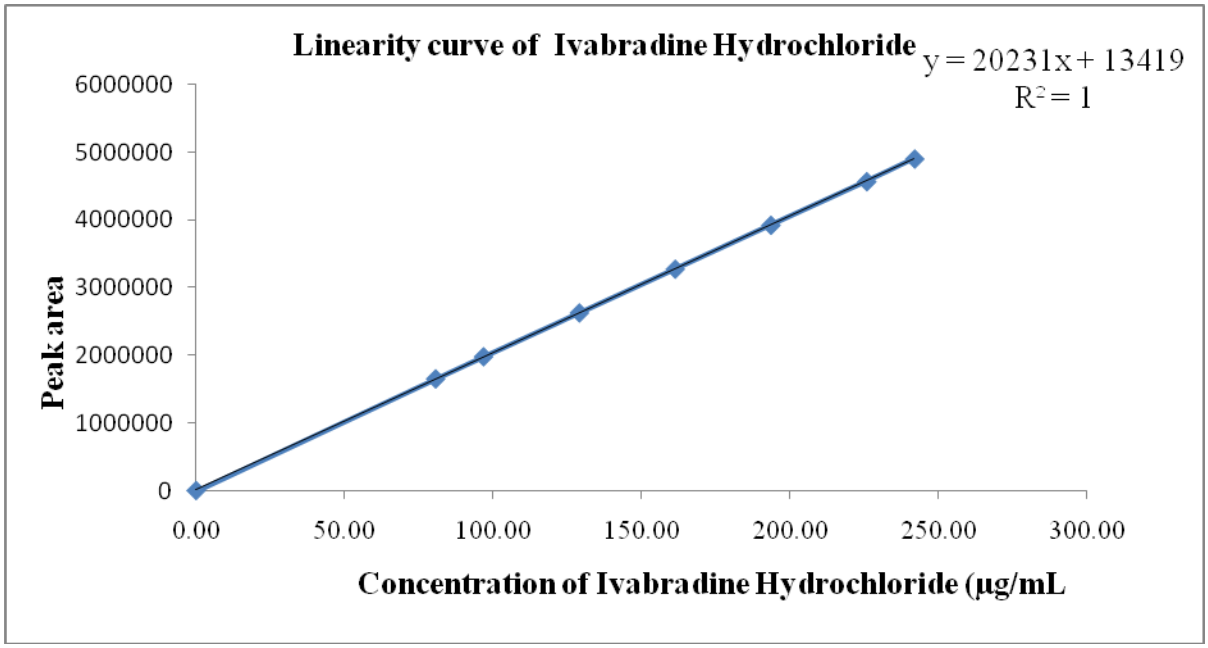

Figure 1: Linearity of ivabradine hydrochloride

Accuracy

Accuracy test was conducted by adding known amounts of analyte to the sample matrix and five different concentrations of test sample were prepared. Duplicate injection was made for each concentration level. Concentration values were calculated from the corresponding peak areas for five concentrations. Percent recoveries or percent of target were calculated. The accuracy test result is presented in Table 3 with acceptance criteria. The accuracy was found to be 100.37%.

Table 3: Result of Accuracy

% of Nominal Value	Weight		Peak area of Sample	Average peak area of sample	Peak area of Std	Recovery	%Recovery	Limit
	API (mg)	Placebo (mg)						
80%	32.5	450.0	2657778	2658367	3276949	32.7	100.59	98.0% to 102.0%
			2658955					
90%	36.1	450.0	2925734	2919162		35.9	99.45	
			2912589					
100%	40.0	450.0	3218853	3222239		39.6	99.07	
			3225625					
110%	44.2	450.0	3659369	3653968		44.9	101.67	
			3648566					
120%	47.8	450.0	3930549	3928767		48.3	101.08	
			3926985					
Mean							100.37	
SD							1.09	
%RSD							1.090%	

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Precision

Repeatability

One analyst (analyst 1) conducted assay by using six samples. Area from duplicate injections was measured for each of six sample preparations. Concentration was calculated from the corresponding area for six samples. Percent relative standard deviations were calculated for using the following relationship:

%RSD = (Standard Deviation ÷ Mean Concentration) x 100%

This percent relative standard deviation proves the repeatability of the method. The data is presented in Table 4 with acceptance criteria. % RSD for repeatability of the method was found to be 0.505%.

Table 4: Result of Repeatability

Sample	STD (mg)	Peak area of Sample	Average peak areas of Sample	Peak area of Std	Assay, (mg per Tablet)	%RSD	Limit
1	55.1	3269144	3267313	3276949	7.63	**0.505**	NMT 2.0%
		3265482					
2		3225986	3225736		7.66		
		3225485					
3		3200828	3206740		7.66		
		3212652					
4		3201191	3204088		7.67		
		3206985					
5		3245148	3249923		7.73		
		3254698					
6		3156393	3163124		7.62		
		3169855					
Average of Assay (mg per tablet)					7.66		

Intermediate precision

Separately a second analyst checked the assay of six sample of the same batch as the analyst 1. Area from duplicate injections was measured for each of six sample preparations. Concentration was calculated from the corresponding area for six samples. The precision of analyst 2 were combined with that of the analyst 1 (n=12) and the combined relative standard deviation (RSD) was calculated to check the intermediate precision. The data is presented in Table 5 with acceptance criteria. The intermediate precision of the method was found to be 0.76% (Table 5).

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Table 5: Result of Intermediate precision:

Sample		STD (mg)	Peak area of Sample	Average peak areas of Sample	Peak area of Std	Assay, mg per tablet	%RSD	%RSD of 12 sample	Limit (%)
Analyst 1	1	40.3	3269144	3267313	3276949	7.63	0.505	**0.760**	NMT 2.0
			3265482						
	2		3225986	3225736		7.66			
			3225485						
	3		3200828	3206740		7.66			
			3212652						
	4		3201191	3204088		7.67			
			3206985						
	5		3245148	3249923		7.73			
			3254698						
	6		3156393	3163124		7.62			
			3169855						
Analyst 2	1	41.0	3356445	3341499	3375958	7.64	0.660		
			3326552						
	2		3292477	3294531		7.58			
			3296584						
	3		3298655	3297621		7.64			
			3296586						
	4		3199564	3203082		7.60			
			3206599						
	5		3299996	3282971		7.52			
			3265945						
	6		3269852	3263420		7.54			
			3256987						

Specificity

Specificity of the analyte was determined from the visual observation of different chromatograms. The chromatograms recoded from blank injection, placebo injection, standard injection and test sample injection were used to find the specificity of target analyte. Necessary chromatograms are presented below:

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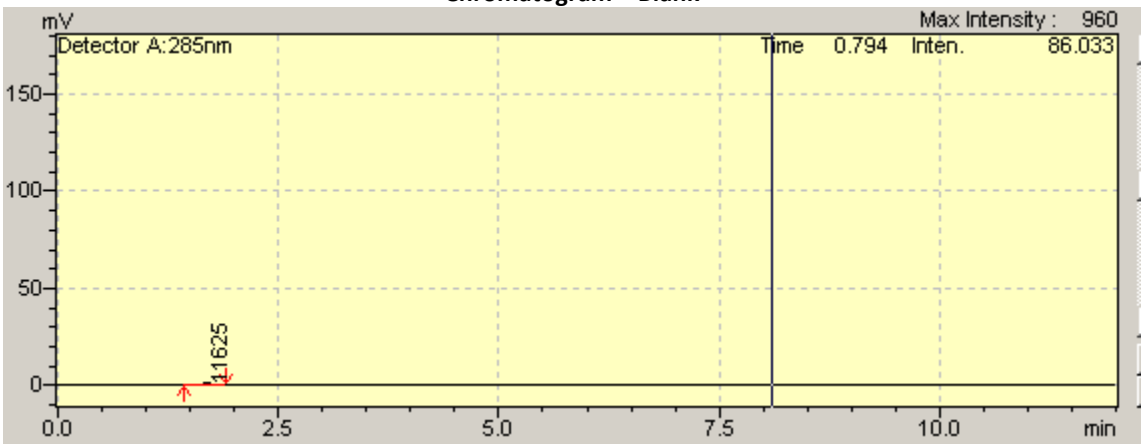
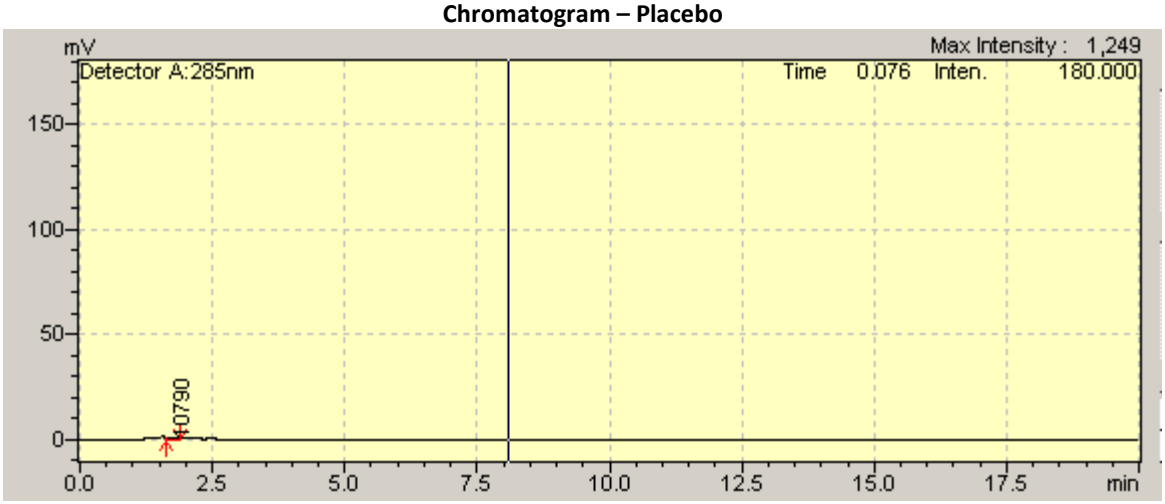
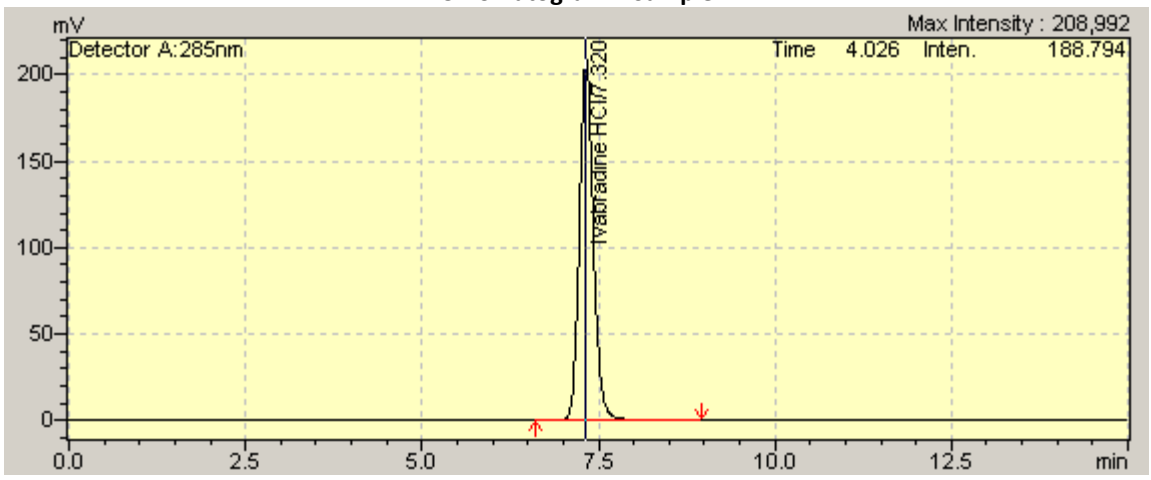
Chromatogram – Blank



Chromatogram – Placebo



Chromatogram – Sample



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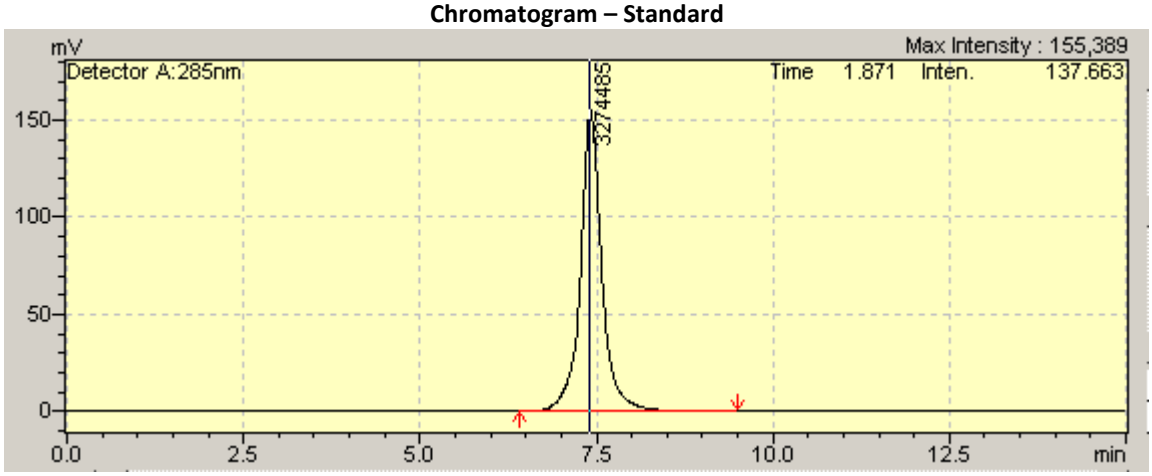
Chromatogram – Standard



Robustness

The robustness of a method is its ability to remain unaffected by small changes. Robustness study was performed by making slight variations in flow rate, amount of methanol and temperature. 100% of target concentration was used in this study. The results of robustness in the present method showed no significant changes which are summarized in Table 6. As the changes are not significant we can say that the method is robust

Table 6: Results for robustness test

Parameters	Changes	% Recovery
Flow Rate (ml/min)	0.9	99.7
	1.1	99.6
Column Temperature (^{0}C)	32	99.6
	35	99.5
Methanol Variation	15%	99.6
	17%	99.6

Analysis of Market products

The proposed method was used to determine the potency of commercially available tablets (one brand in two different strength) containing ivabradine. Six replicate determinations (n=6) were carried out. Potency of the market products was within 98.7%-100.14%.

CONCLUSION

From the above test parameters it is proved that the System Suitability, Linearity and Range, Accuracy, Precision (Repeatability, Intermediate), Limit of Quantitation and Specificity were found within the required range. Therefore this method is suitable for the assay of Ivabradine hydrochloride in bulk and tablet dosage forms.

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