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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IVABRADINE HYDROCHLORIDE BY USING RP-HPLC

Ashwini S. Pundkar*[1] and Pradyumna V. Vyas[1]

[1*]Rajesh Bhaiyya Tope College of Pharmacy, Aurangabad, Maharashtra, India.

ABSTRACT

A High Performance Liquid Chromatography (HPLC) have been developed for the estimation of Ivabradine Hydrochloride in bulk drug and pharmaceutical dosage form. The method is carried out using C18 column 250×4.6mm i.e. particle size 5µm and mobile phase consisting of Methanol: Water (50:50), at flow rate of 0.6ml/min. The column temperature is ambient. Eluents were monitored by UV detector set at 286nm. The method was statistically validated in terms of linearity, accuracy, precision and robustness in accordance with ICH guidelines Linear regression analysis data for the calibration plot showed that there was a linear relationship between response and concentration in the range of 08µgm/ml to 56µgm/ml and the correlation coefficient is 0.9998. Literature survey reveals analytical methods for the estimation of Ivabradine Hydrochloride from pharmaceutical dosage forms and also in biological fluids. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of Ivabradine Hydrochloride in bulk drug and tablet.

KEYWORDS

Ivabradine hydrochloride, Method Development, UV and HPLC validation.

Author for Correspondence:

Ashwini S. Pundkar,

Rajesh Bhaiyya Tope College of Pharmacy,

Aurangabad, Maharashtra, India.

Email: pundkar.ashwini@gmail.com

INTRODUCTON

Ivabradine hydrochloride is a novel heart rate lowering medicine for the symptomatic management of stable angina pectoris and symptomatic chronic heart failure. Ivabradine brand name corlanor was approved by the FDA in April 2015 for the treatment of chronic heart failure in patients with an ejection fraction of <35%, in sinus rhythm with resting heart rate > beats per minute, who are not on beta blockers due to contra indicators or already receiving maximum beta blocker dose. It is 3-[3-({[(7S)-3, 4 dimethoxybicyclo [4.2.0] octa-1, 3, 5-trien-7yl]

methyl} (methyl) amino) propyl]-7-8-dimethoxy-2, 3, 4, 5-tetrahydro-1-H-3-benzazepin-2-one.

The aim of the present work was to develop a simple and economic liquid chromatographic method that would be suitable for determination of Ivabradine and its impurities in bulk and dosage form. The proposed method is found to be simple, accurate, reproducible and suitable for routine determination of Ivabradine from its pharmaceutical dosage form.

Molecular formula

$C_{27}H_{36}N_2O{\cdot}HCL$

Molecular weight

468.585g/mol

Solubility and Description

White colored amorphous powder. Soluble in methanol and water

Melting Point

135°C to 140°C

Pharmacokinetic Data

Storage

Stored under cool and dry place.

MATERIAL AND METHODS

The drug sample of Ivabradine HCL obtained from Lupin research center, Aurangabad. The formulation (Ivabrad-5mg tablet) used was purchased from local pharmacy. Fischer Ltd. Mumbai, India, supplied HPLC grade methanol and water. High Pressure Liquid Chromatograph (Thermo scientifics and software is Chrome quest) with auto sampler injector, variable wavelength programmable UV-Vis detector 1800 Shimadzu Corporation, Tokyo, Japan was used. The chromatography column used was a reversed phase C_{18} column (250×4.6 mm i.e. particle size 5µm). A mixture consisting of methanol: water (50:50) was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 0.6ml/min. Detection was carried out at 286nm ambient temperature.

Experimental work

Drug Identification Test

Melting Point

Melting point of the drug was determined by Melting point apparatus with the help of capillary tube.

Solubility Test

10mg of Ivabradine hydrochloride was weighed accurately and transfer into a 10ml volumetric flask. Few drops of water were added and shake thoroughly.

Determination of wavelength (λ max)

The sensitivity of the HPLC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for the drugs to be detected. A UV spectrum of Ivabradine was recorded between 200-400nm.

Method of HPLC for Ivabradine

Selection of Mobile Phase

A different combination of Methanol and Water was on the basis of its polarity. The solvent system was optimized in order to provide a good performance of the assay. Finally, Mobile Phase which containing Methanol and Water (50:50) was selected for further analysis.

Selection of Detection wavelength

The detection wavelength was measured by running the 20µg/ml solution of Ivabradine in Mobile Phase, and the wavelength of maximum absorption was selected as 286nm.

Preparation of standard stock solution

10mg of Ivabradine HCL weighed accurately and transferred to 100ml volumetric flask containing a mixture of methanol and water in the ratio of 50:50v/v. The volume is made up to mark using the same mixture of solvent to obtain the resulting solution of 100µg/ml. The absorbance of latter was recorded using UV visible spectrophotometer in ranges 200-400nm.

Preparation of working solution

10 mg of Ivabradine HCL weighed accurately and transferred to 100ml volumetric flask containing a mixture of methanol and water in the ratio of 50:50v/v. The volume is made up to mark using the same mixture of solvent to obtain the resulting solution of 100µg/ml. Then 0.8ml, 1.6 ml, 2.4 ml, 3.2ml, 4.0ml, 4.8ml and 5.6ml to give concentration of 8, 16, 24, 32, 40, 48 and 56ppm.

System suitability testing

Preparation of working solution

From freshly prepared standard stock solution (100µg/ml), 1.0ml stock solution was pipette out and diluted up to 10ml to obtain consequential

solution of 10µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 4.30min. Three replicates of this solution were injected and result were recorded for RT, area, tailing factor, theoretical plates, SD, % RSD were calculated.

RP-HPLC method development

Preparation of standard stock solution

10mg of Ivabradine HCL weighed accurately and transferred to 100ml volumetric flask containing a mixture of methanol and water in the ratio of 50:50v/v. The volume is made up to mark using the same mixture of solvent to obtain the resulting solution of 100µg/ml. The absorbance of latter was recorded using UV visible spectrophotometer in ranges 200 400nm.

Linearity

From stock solution 0.8ml, 1.6ml, 2.4ml, 3.2ml, 4.0ml, 4.8ml, and 5.6 ml of solution were pipette out in 10ml volumetric flask and volume was made up to the mark with mobile phase to get final concentration 8, 16, 24, 32, 40, 48, 56µg/ml.

Sample were injected and peaks were recorded at 286 nm as the calibration curve was constructed between concentration of drug verses peak area. Results were recorded for equation of line, correlation coefficient and intercept were determined.

$$Y = mX + C$$

Equation 1 Equation of line

Where,

Y - Area

X - Unknown concentration

M - Slope of graph

C - Intercept

Precision

From the calibration range three QC standard defined viz. 10, 30 and 50µg/ml as LQC, MQC and NQC respectively. The solution for Qc standard was prepared by dilution stock solution (100µg/ml) of 1.0, 3.0 and 5.0ml solutions up to 10ml. Area of each QC standard was recorded for intraday and inter day precision in seven replicate as per ICH Q2R1 guidelines.

Intra-day precision

Three different concentration (10µg/ml, 30 µg/ml, 50µg/ml) were analyzed three times on the same day and %R.S.D was calculated.

Acceptance criteria precision

The relative standard deviation peak area should not be more than 2% for test.

Percent Accuracy

%Accuracy was determined using observations of precision study using following formula.

% Accuracy= Mean measured concentration/ nominal x 100

Robustness

The organic concentration of mobile phase was changed in (±2 ml). The flow rate was changed in (±0.05) the effect of the results were examined. It was performed using 16µg/ml solution of Ivabradine Hcl in triplicate.

Detection Limit

Based on the standard error of the predicted y value for each x in a regression, the detection limit *(*DL*)* was calculated as,

$$LOD = \frac{3.3 \times STEYX}{SLOPE}$$

Quantitation limit

Based on the standard error of the predicted y value for each x in a regression, the quantitation limit *(*QL*)* was calculated as,

$$LOQ = \frac{3.3 \times STEYX}{SLOPE}$$

%Recovery

Preparation of stock from API

Accurately weighed 10 mg of Ivabradine Hcl API was added in volumetric flask 100 ml containing some amount of mobile phase and volume was made up to the mark using mobile phase from stock solution aliquots of 1ml were precipitated out and kept in three different 10 ml volumetric flasks, cleaned previously and diluted up to the mark to obtain resultant solution of 100µg/ml respectively. The resultant solution was filtered through 0.45µ membrane filtered and sonicated for cycle each of 10 min. This solution injected for given chromatographic system and area was determined in each case.

Preparation of test solution for %recovery by spike method

Percentage recovery is determination of percent purity of given analyte in finished product. The accuracy of the method was determined by calculating recoveries of Ivabradine Hcl by the standard addition method. Known amount of standard solution of Ivabradine Hcl (10µg/ml) were

added to a simple solution of Ivabradine Hcl (13, 16 and 19µg/ml) representing 80, 100, 120 levels. Each of this solution was injected in triplicate and means area in each case was determined. The mean area of standard solution (10µg/ml) was substracted from letter to get the actual area corresponding to the sample solution at each level. The equivalent mean measured concentration for each level was determined. The finally percentage recovery was calculated from mean measured concentration and nominal concentration recovered amount was within the limit as per compendia standards for Ivabradine Hcl.

$$\%\text{recovery} = \frac{\text{sample area}}{\text{standard area}} \text{x} \frac{\text{standard conc.}}{\text{sample conc.}} \text{x} 100$$

RESULTS AND DISCUSSION

Drug Identification Test

Melting Point

Melting point observed by capillary method using digital melting point apparatus and was observed to be 138ºC. (Reported M.P. 135ºC -140ºC).

Solubility Test

Soluble in water and methanol.

Determination of wavelength (λ max)

Initially 10ug/ml solution of Ivabradine Hcl was prepared in the mixture of methanol: water (50:50, 70:30, 90:10). This solution was subjected to UV analysis to determine the absorption maxima (max). The UV spectrum obtained in each case were shown in the absorbance values obtained at different concentration of different mobile phase were as tabulated in table and respectively. From the result obtained in each case the absorbance value at wavelength 286 nm was found to be 0.2 (maximum absorbance).

Characterization by IR

IR spectroscopic technique was used to determine functional group present in organic molecules. Results obtained in terms of wave number corresponds to different functional group of drug as given in table. Result obtained showed the corresponding functional group present in Ivabradine HCL. This confirmed the API Ivabradine HCL.

Selection of analytical wavelength

Using appropriate dilution of standard stock solution, the solutions were scanned in order to get good results. The wavelength selected should be such that at wavelength the absorptivity of components should be as large as possible. So the wavelength chosen was 286nm for Ivabradine.

HPLC METHOD DEVELOPMENT AND VALIDATION

Chromatographic condition

RP-HPLC method was developed for Ivabradine, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. A different combination of methanol and water was on the basis of its polarity. Finally, mobile phase which containing Methanol: Water (50:50) was selected for further analysis. These chromatographic conditions gave a retention time 4.5 minutes.

Chromatograph for Ivabradine

Preparation of standard stock solution

10 mg of Ivabradine HCL weighed accurately and transferred to 100ml volumetric flask containing a mixture of methanol and water in the ratio of 50:50v/v. The volume is made up to mark using the same mixture of solvent to obtain the resulting solution of 100µg/ml. The absorbance of latter was recorded using UV visible spectrophotometer in ranges 200-400nm.

All parameters found within limit as per ICH guidelines. Therefore, method passed from system suitability test for selected chromatographic condition.

RP-HPLC method validation

Linearity

The linearity of an analytical procedure is its ability to elicit test results that are proportional to the concentration of analyte in sample. From the stock standard solution, aliquots of 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 and 5.6ml were taken in 10ml volumetric flasks and diluted up to the mark with mobile phase such that to obtained concentration of Ivabradine Hcl in the range 8-56µg/ml. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area verses the drug concentration

From correlation coefficient (r^2) obtained, it was found that there was a linear correlation between the concentration and observed area. The linearity was

found within the range of 8-56µg/ml. hence this method could be used to determine the concentration of Ivabradine HCL quantitatively within given range

Precision

The precision of an analytical method is the degree of agreement among individual test result when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurement.

From the calibration range three QC standards were defined viz. 10, 30 and 50µg/ml. The solution for QC standard were prepared by diluting stock solution (100µg/ml) of 1.0, 3.0 and 5.0ml solutions up to the 10l using mobile phase (methanol: water 50:50). Absorbance of each QC standard were recorded from intra and inter day precision as per ICH guidelines Q2R1.

Mean area of seven replicate injection of each QC standard was then statistically analyzed to determined SD and %RSD table. Result showed that method is precise for selected calibration range as %RSD for mean area of each QC standard was less than

2%. Hence this method might be use precisely for the analysis of bulk Ivabradine Hcl Consequently the method was set to precise for selected calibration range.

Recovery

Accuracy studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug 80, 100 and 120% was added and theist recovery was analyzed.

Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±2 ml/min^{-1}) proportion and the flow rate was varied by (±0.05ml/min^{-1}) optimized chromatographic condition. The results of robustness studies. Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Result obtained from this study showed that robustness lied within the limit so proposed method was found to be robust.

Detection of limit

The LOD is the lowest limit that can be detected based on the standard error of the predicted y value for each x in a regression. The limit of detected (LOD) may be expressed as:

LOD=3.3×average of S.D./ slope

Where,

Average of S.D = 3402.7

Slope= 39586

Limit of detection= 0.28 µg/ml

The LOD of Ivabradine HCL was found to be 0.28µg/ml, analytical method that concluded.

Quantitation of limit

The LOQ is the lowest concentration that can quantitatively measure based on S.D. deviation of the response and the slope. The quantitation limit (LOQ) may be expressed as:

LOQ = 10×average of S.D./ slope

Where,

Average of S.D= 3402.7

Slope= 39586

Limit of quantitation= 0.85µg/ml

The LOQ was found to be 0.85µg/ml, analytical method that concluded

Recovery

Percentage recovery is determination of percent purity of given analyte in finished product. The accuracy of the method was determined by calculating recoveries of Ivabradine Hcl by the standard addition method. Known amount of standard solution of Ivabradine Hcl (10µg/ml) were added to a simple solution of Ivabradine Hcl (13, 16 and 19 µg/ml) representing 80, 100, 120 levels. Each of this solution was injected in triplicate and means area in each case was determined. The mean area of standard solution (10µg/ml) was substracted from letter to get the actual area corresponding to the sample solution at each level. The equivalent mean measured concentration for each level was determined. The finally percentage recovery was calculated from mean measured concentration and

nominal concentration recovered amount was within the limit as per compendia standards for Ivabradine Hcl Table No.9.

Result obtained from this study that percent recovery lied within the limit so proposed method was found to be accurate

SUMMARY AND CONCLUSION

The present study was aimed at developing a sensitive, precise, and accurate HPLC method for analysis of Ivabradine HCL bulk drug in pharmaceutical dosage forms. In order to analysis of the component peaks, mixture of methanol and water in different combination were tested as mobile phase on a C18 stationary phase. A mixture of methanol and water in a proportion of 50:50 v/v was proved to be the most suitable of all combination since the chromatographic peaks were defined and resolved and almost free from tailing. The retention time obtained for Ivabradine HCL was 4.5 min each of the sample injected three times and reproducible retention

System suitability parameter was studied with six replicate standard solution of the drug and calculated parameters were within the acceptance criteria the tailing factor and the number of theoretical plates in acceptable limit. The peak area of Ivabradine HCL was reproducible as indicated by low coefficient of variation. A good linear relationship (r^2= 0.997) was observed between the concentration of Ivabradine HCL and respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was Y = 39586x + 7091.

(Where Y gives peak areas and X is the concentration of drug) When Ivabradine HCL solution contains 10, 30 and 50 µg/ml was analyzed the proposed method for finding out intra and inter day variations %RSD was observed. The high recovery values obtained from the dosage form by the purposed method indicated accuracy. The absence of addition peaks indicated noninterference of common excipients used in tablets. The drug contain in tablets was quantified using analytical method. The tablets were found to contain an average of 98 - 102% of the labeled amount of the drug. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and % assay. This indicates that the present method is robust. The lowest value of LOD and LOQ was obtained by the proposed method indicated its sensitivity. The standard solution of the drug was stable of to 24 hours as the different in present is within acceptable limit. Hence the author proposed that the present HPLC method was sensitive and reproducible for the analysis of Ivabradine HCL in pharmaceutical dosage forms with short analysis time.

1	Bioavailability	40%
2	Protein Binding	70%
3	Metabolism	Liver and Intestine
4	Biological half life	2hr.
5	Dose	2.5, 5 and 7.5mg
6	Excretion	Excreted unchanged in urine
7	Category	Anti- Hypertensive

Table No.1: Robustness variation

S.No	Condition	Normal	Variation 1	Variation 2
1	Mobile phase	50:50	48:52	52:48
2	Flow rate (ml/min)	0.6ml	0.55	0.65

Table No.2: Interpretation of IR

S.No	Group	Standard peak	Observed peak
1	C-H bending	900-690	803.37
2	C-O stretching	1250 and 1040	1025
3	C-F stretching	1250-1100	1126.678
4	C-O stretching	1450	1405.96
5	C=C stretching	1608	1594.12
6	C=O stretching	1685	1665

Table No.3: Observations of UV spectrum

S.No	Wavelength	Absorbance
1	286.20000	0.20544
2	355.40000	-0.00729
3	312.20000	-0.00653

Table No.4: System suitability parameter

S.No	Name	Retention time(min)	Area	No. of theoretical	Tailing factor
1	Ivabradine Hcl	4.30	405290	4080	1.52

Table No.4: Linearity data of Ivabradine Hcl

S.No	Name	Retention Time(min)	Area	%Area	No. of Plates	Theoretical Tailing Factor
1	Ivabradine	4.20	1343906	100%	4256	1.38

Table No.5: Linearity data for Ivabradine HCL

S.No	Conc (µg/ml)	Mean Area*
1	8	312458
2	16	633981
3	24	937307
4	32	1350464
5	40	1570257
6	48	1897733
7	56	2214740

S.No	Name	Retention time	Area	%Area	Theoretical Plate	Tailing factor
1	Ivabradine	4.37	42695	100%	3958	1.51

S.No	Name	Retention Time(min)	Area	%Area	No. of Theoretical Plates	Tailing Factor
1	Ivabradine	4.30	409960	100%	4864	1.22

Table No.6: Results of Inter and Intraday precision study of Ivabradine HCL

S.No	Conc	Intraday precision			Inter day precision		
		Mean area	SD	%RSD	Mean area	SD	%RSD
1	10	426025.00	481.93	0.11	420910.67	7219.11	1.71
2	30	1272838.33	4121.28	0.32	1263579.56	16011.36	1.26
3	50	2105512.67	5311.17	0.25	2080430.44	43453.10	1.32

S.No	Name	Retention	Area	Theoretical plates	Tailing Factor
1	Ivabradine	4.19	1131888	4376	1.42

S.No	Retention	Area	Theoretical plates	Tailing Factor
1	4.30	130315	4605	1.46

S.No	Retention	Area	Theoretical plates	Tailing Factor
1	4.31	1407542	4259	1.49

Table No.7: Result of % accuracy data

S.No	Conc (µg/ml)	Mean Area Conc. (%)	Measured% accuracy
1	29	1143405	98.96
2	32	1296958	101.81
3	35	1410487	101.2

S.No	Name	Retention Time(min)	Area	Theoretical Plate	Tailing factor
1	Ivabradine	4.08	650473	4145	1.45

S.No	Retention Time	Area	Theoretical Plate	Tailing factor
1	4.05	648922	4648	1.45

S.No	Name	Retention Time	Area	Theoretical Plate	Tailing factor
1	Ivabradine	4.44	651550	4632	1.42

Table No.8: Result of Robustness study of Ivabradine Hcl

S.No	Parameter	Conc. (µg/ml)	Amount of detected mean area	R.T.	% assay Limit (98-102%)
1	Chromatogram of mobile Phase of change 48:52	16	647080	4.05	101.04
2	Chromatogram of mobile phase of change 52:48	16	649528	4.44	101.431
3	Chromatogram of flow Change 0.65ml	16	652470	4.08	101.895
4	Chromatogram of flow Change 0.55ml	16	652530	4.55	101.905

Table No.9: Result of Recovery study of Ivabradine HCL

S.No	%recovery	Level std	Amt. sample	Amt. area	Amt. recovered	%recovery
1	80	16	13	509424	12.68	97.34
2	100	16	16	662977	16.56	102.88
3	120	16	19	776506	19.43	101.38

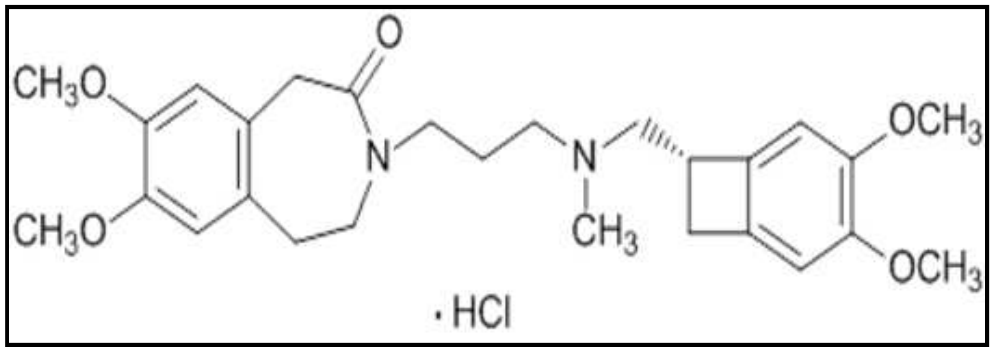

Figure No.1: Structure of the Ivabradine hydrochloride

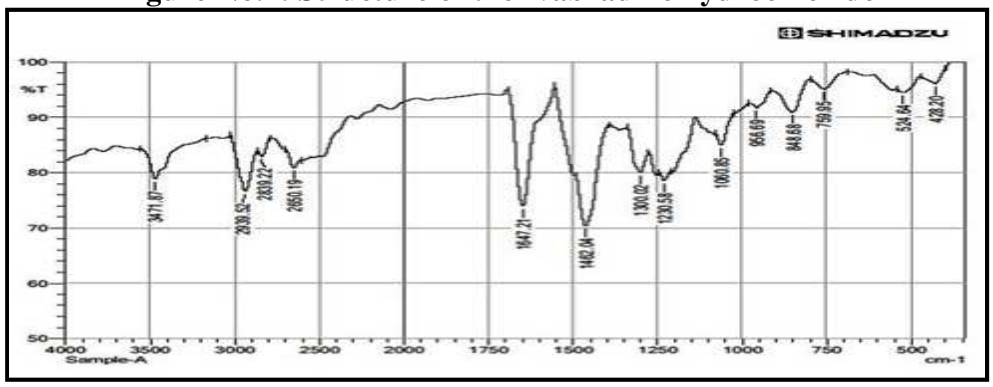

Figure No.2: IR spectrum of Ivabradine HCL

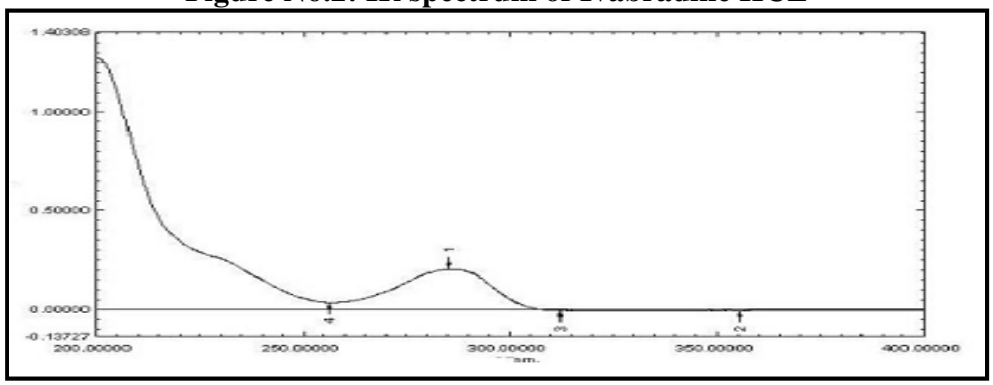

Figure No.3: UV spectrum of Ivabradine HCL

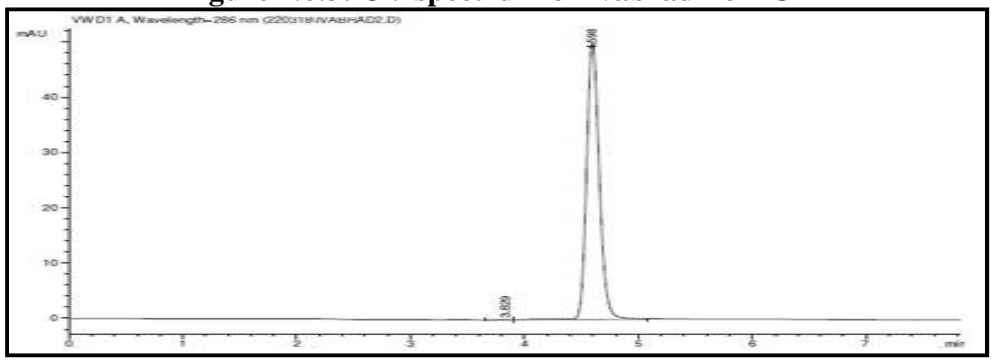

Figure No.4: Trial 3(Method Optimization)

Retention Time: 4.5min

Area: 390.7

Theoretical Plate: 9006

Tailing Factor: 0.83

System Suitability

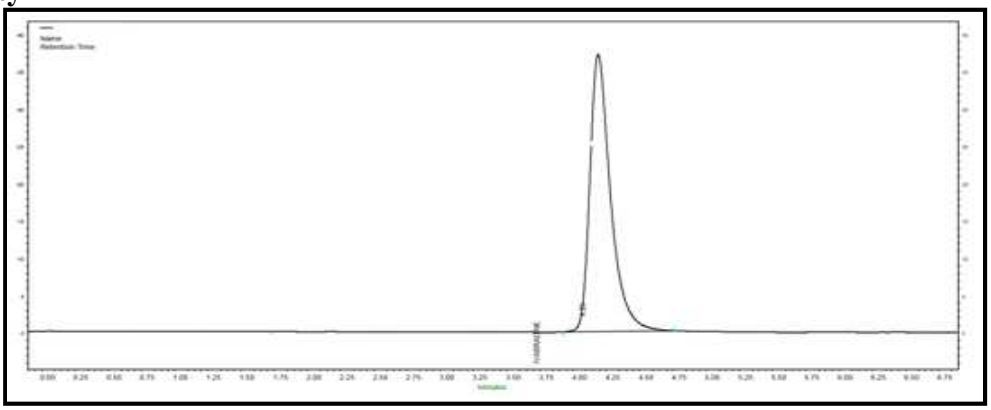

Figure No.5: Chromatogram of system suitability

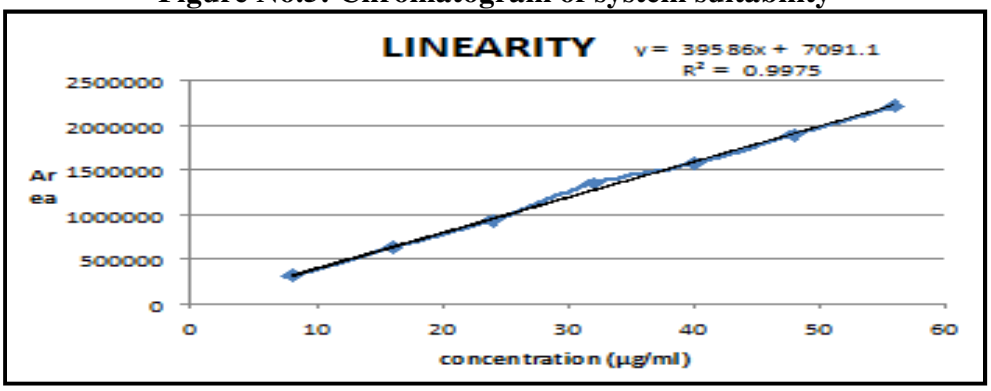

Figure No.6: Calibration curve for Ivabradine

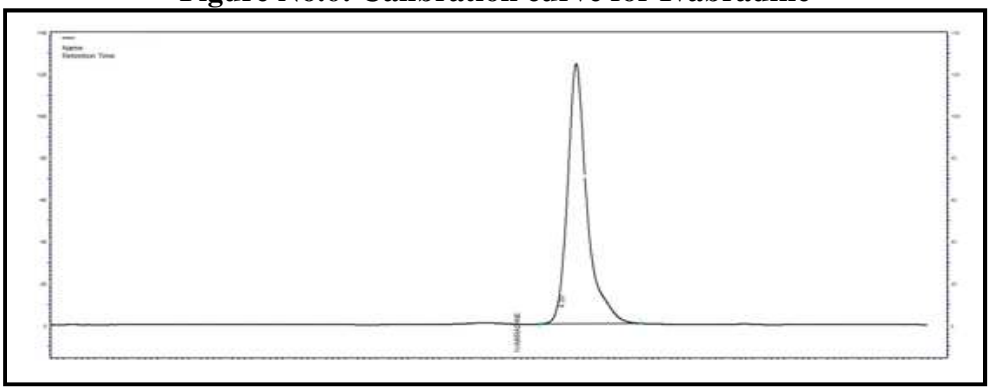

Figure No.7: Chromatogram of Linearity

Intra-day Precision

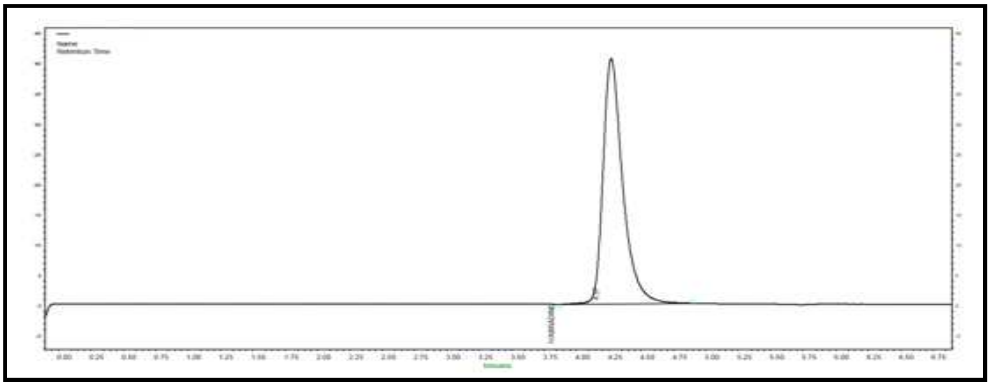

Figure No.8: Chromatogram of Intraday precision

Inter day

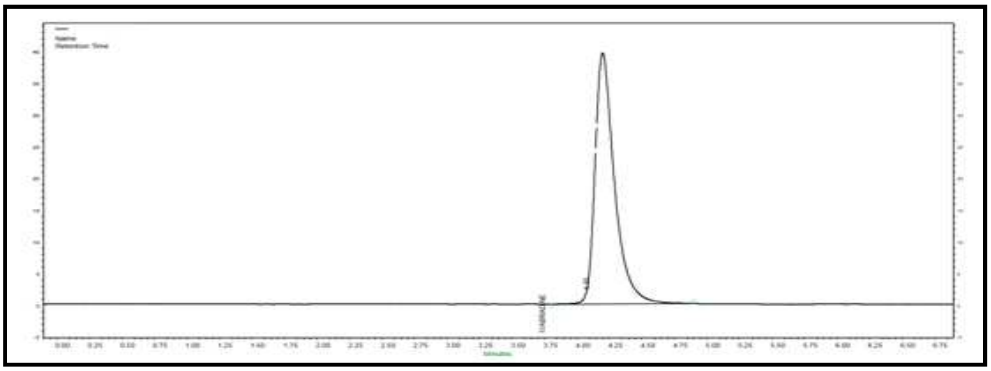

Figure No.9: Chromatogram of Inter-day precision

Accuracy 80%

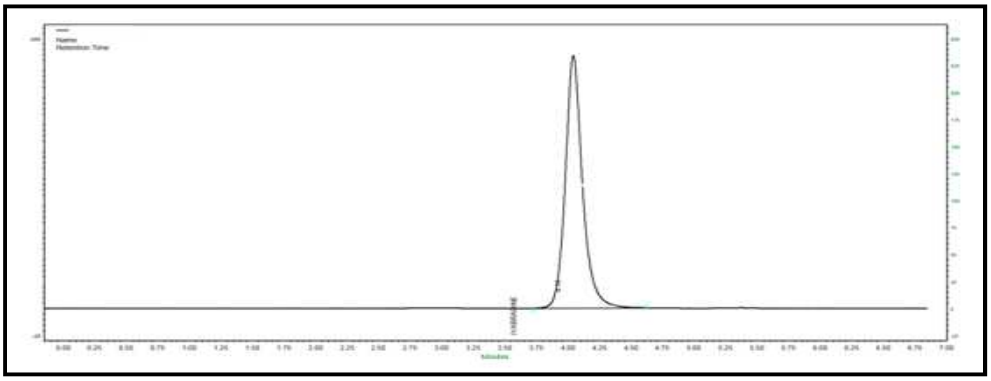

Figure No.10: Chromatogram of accuracy 80%

Accuracy 100%

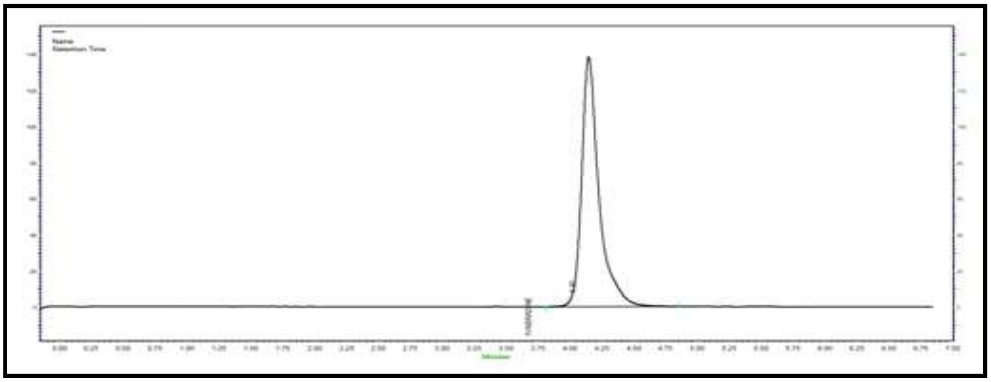

Figure No.11: Chromatogram of accuracy 100%

Accuracy 120%

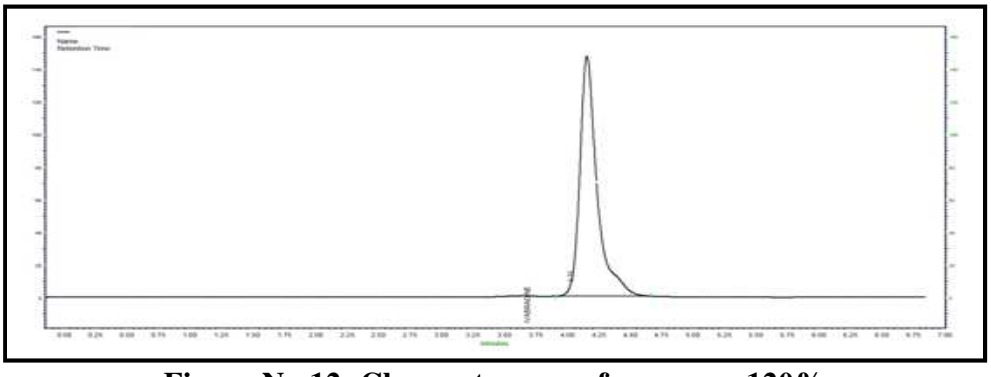

Figure No.12: Chromatogram of accuracy 120%

Flow rate change 0.65ml

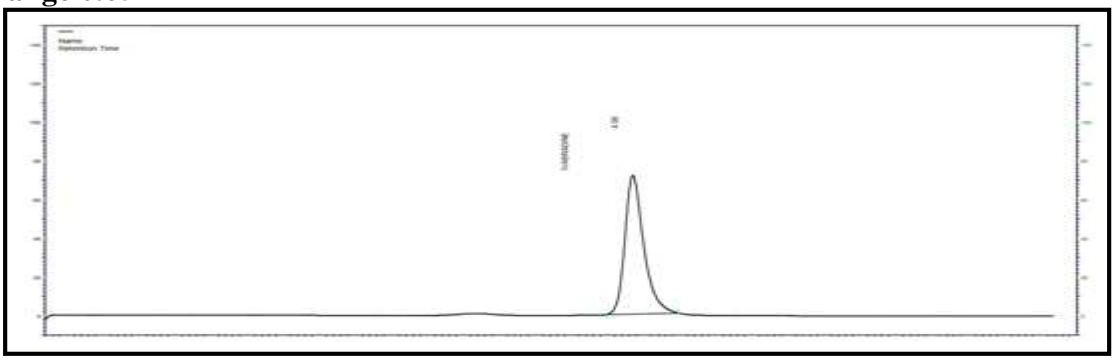

Figure No.13: Chromatogram of Flow rate change 0.65ml

Flow rate change 0.55ml

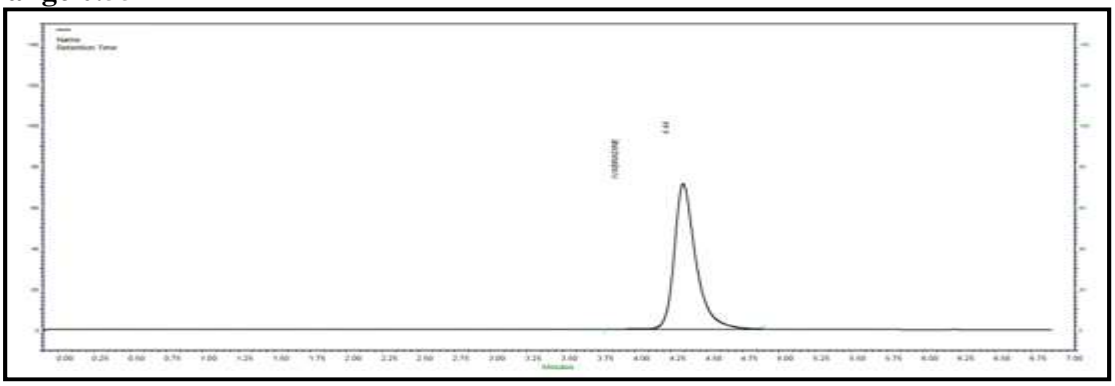

Figure No. 14: Chromatogram of Flow rate change 0.55ml

Composition change 48:52

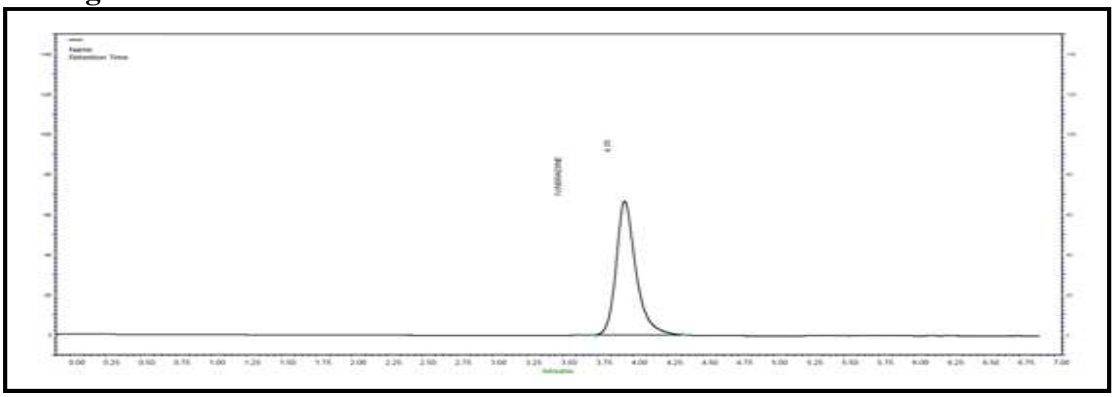

Figure No.15: Chromatogram of composition change 48:52

Composition change 52:48

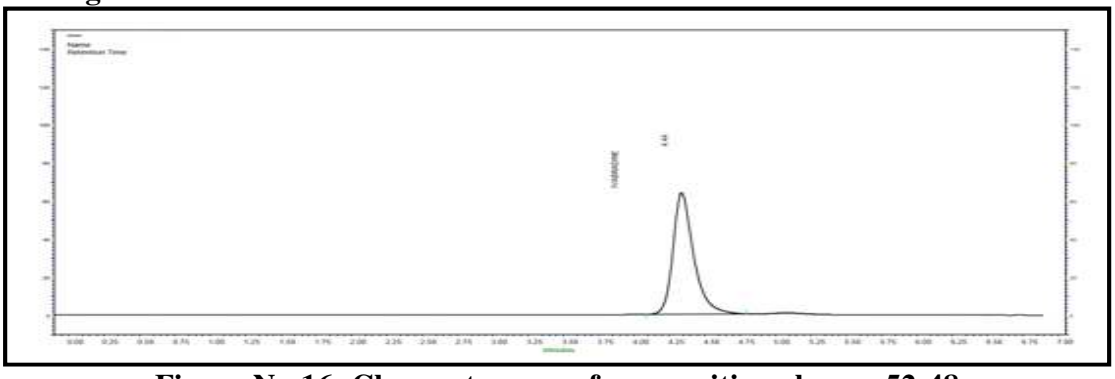

Figure No.16: Chromatogram of composition change 52:48

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC methods have been developed and validated for the routine analysis of Ivabradine HCL API and tablet dosage forms. HPLC method was suitable for the simultaneous determination of Ivabradine HCL formulations without interference of each other. The developed method was recommended for routine and quality control analysis of the investigated drugs in component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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