



Research Article

ISSN: 0975-248X  
CODEN (USA): IJPSPP



## **Validated RP-HPLC Method for the Determination of Ivabradine Hydrochloride in Pharmaceutical Formulation**

**Md. Muzaffar-ur- Rehman<sup>1\*</sup>, G. Nagamallika<sup>2</sup>**

<sup>1</sup>Department of Pharmaceutical Analysis, St. Mary's College of Pharmacy, Secunderabad-500025, Telangana, India

<sup>2</sup>Department of Pharmaceutical Chemistry, St. Paul's College of Pharmacy, Turkayamjal (V), Nagarjuna Sagar Road, R.R. Dist, Hyderabad-501 510, Telangana State, India

Copyright © 2017 Md. Muzaffar-ur- Rehman *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

### **ABSTRACT**

A simple, rapid, precise, and accurate RP-HPLC method for the estimation of Ivabradine Hydrochloride an anti-anginal agent, both as a bulk drug and in pharmaceutical formulation was developed. The chromatographic separation was achieved on a Thermosil C<sub>18</sub> 150 × 4.5 mm, 5µm column by using a mobile phase containing a mixture of methanol and phosphate buffer pH 6.5 in the ratio of 65:35 % v/v at a flow rate of 1ml/min and at an ambient temperature. The detection was monitored at a wavelength of 265nm. A clear chromatographic peak was identified with the retention time of 4.36 min and tailing factor of 1.23. The developed method was validated according to ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method shows a good linear relationship with correlation co-efficient of more than 0.992 in the concentration range of 30µg-150µg. The method showed mean % Recovery of 100.4% and %RSD for repeatability and intermediate precision was less than 2%. The proposed method can be used successfully for the quantitative determination of Ivabradine HCL in pharmaceutical dosage forms.

**Keywords:** Ivabradine hydrochloride, ICH guidelines, Estimation, Capsules, RP-HPLC.

**DOI:** 10.25004/IJPSDR.2017.090505

**Int. J. Pharm. Sci. Drug Res. 2017; 9(5): 228-233**

\*Corresponding author: Mr. Md. Muzaffar-ur- Rehman

Address: Department of Pharmaceutical Analysis, St. Mary's College of Pharmacy, Secunderabad-500025, Telangana, India

Tel.: +91-8686294437

E-mail ✉: [m.muzaffar687@gmail.com](mailto:m.muzaffar687@gmail.com)

**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Received:** 22 July, 2017; **Revised:** 07 August, 2017; **Accepted:** 08 August, 2017; **Published:** 15 September, 2017

### **INTRODUCTION**

Chemically Ivabradine is 3-[3-((7s)-3,4-dimethoxy bicyclo[4.2.0]octa-1,3,5-trien-7yl)-methyl](methyl)amino propyl]-7,8 dimethoxy-2,3,4,5 tetrahydro-H-3-benzazepin-2-one, having a molecular formula of C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub> and molecular weight is 468.59 g/mol. It

acts as an anti-anginal agent by lowering the heart rate and has similar effects as that of beta blockers but has lesser side effects compared to them. [1-3] Ivabradine acts selectively by inhibiting IF channels in the heart in a concentration dependent manner. [2]

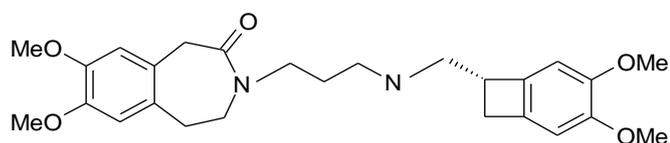


Fig. 1: Chemical structure of ivabradine hydrochloride

A literature search has revealed that very few HPLC methods were reported for assay; stability indicating study both in bulk and tablet formulation. [4-12] LC-MS methods for estimation of Ivabradine in biological samples were published. [13-14] To the best of our knowledge there is no any official method was reported for the assay of Ivabradine hydrochloride in capsules. So, need was felt to develop an analytical method for the estimation of Ivabradine hydrochloride in a capsule formulation. The focus of present study was aimed to develop and validate a new, simple, rapid, accurate, reproducible and economical method for the estimation of Ivabradine hydrochloride in bulk as well as in capsule formulation by RP-HPLC.

## MATERIALS AND METHODS

### Chemicals and reagents

Ivabradine standard drug was obtained as a gift sample from K.P Labs, Hyderabad, India. Methanol (HPLC grade) and water (HPLC grade) and other chemicals of A.R grade were purchased from E. Merk (india) Ltd. worli, Mumbai, India. The 0.22 $\mu$  nylon filters were purchased from advanced microdevices, Chandigarh, India. 0.45 $\mu$  filter paper was purchased from Millipore. Ivabradine hydrochloride capsules were formulated having 150 mg of the drug per capsule.

### Instruments and Chromatographic condition

Liquid chromatographic system (waters) auto sampler separation module 2695, PDA detector 996 was used. The HPLC system was well equipped with empower2 software for data processing. Other equipments like Ascosec digital weighing balance, ADWA Ph meter and EnerTech sonicator were used for sample and standard preparation. The analytical column used was thermosil C-18, 150 x 4.5mm, 5 $\mu$ m packed with a particle size of 5.0 $\mu$ . HPLC grade methanol and phosphate buffer pH 6.5 in the ratio of 65:35% v/v was used as mobile phase at a flow rate of 0.8 ml/min and the injection volume of 10 $\mu$ l, column oven temperature of ambient and UV detection at 265 nm.

### Method Development

Any analytical method was not reported for the estimation of Ivabradine in a capsule formulation. Hence it was significant to start the method development using reverse phase liquid chromatography as it is commonly used and C-18 columns are also available. Different columns were used with different mobile phases during the development of HPLC method suitable for the analysis of Ivabradine hydrochloride in capsule formulation. These involve Nucleosil C<sub>18</sub> 150 x 4.6 mm, 5 $\mu$ m and methanol and water (20:80% v/v), Zodiasil C<sub>18</sub> 150 x

4.6 mm, 5 $\mu$ m and methanol and pH 4.5 buffer (65:35% v/v), Kromosil RPC<sub>18</sub> 150 x 4.6 mm, 5 $\mu$ m and methanol and pH 4.5 buffer (60:40% v/v) and Thermosil C<sub>18</sub>, 150 x 4.5 mm, 5 $\mu$ m and methanol and pH 6.5 phosphate buffer (65:35% v/v). The mobile phase was selected on the basis of sensitivity of the method, time required for the analysis readily available solvents and ease of preparation. Mobile phase were premixed and filtered through a 0.45 $\mu$ m filter. Optimization of mobile phase was taken based on various parameters such as retention time, number of theoretical plates and resolution. Methanol and water (65:35% v/v) was used as diluent.

### Preparation of Ivabradine standard solution

Ivabradine hydrochloride working standard of about 10 mg was weighed accurately and added to 10 ml of volumetric flask and dissolved then diluted to 10 ml with diluent. 1 ml of this solution was taken into 10 ml of volumetric flask and was diluted to 10 ml with the diluent.

### Preparation of Ivabradine sample solution

Twenty capsules were weighed and granules were ground to form fine powder, the powder which is equivalent to 10 mg of drug was accurately weighed and transferred to 10 ml of volumetric flask which was dissolved and diluted to 10 ml with diluent. 1 ml of this solution was taken in 10 ml volumetric flask and made up to the volume with diluent.

### Preparation of phosphate buffer pH 6.5

60.5 g of disodium hydrogen phosphate and 46 g of potassium dihydrogen phosphate were dissolved into water, to this 0.02 M disodium edeate and 20 mg of mercuric chloride were added and diluted to 1000 ml with water.

### Preparation of mobile phase

650 ml of methanol was mixed with 350 ml of phosphate buffer pH 6.5. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 $\mu$ m filter under vacuum.

### Procedure

A mobile phase of methanol and phosphate buffers pH 6.5 in the ratio of 65:35% v/v was found to be the most suitable mobile phase for ideal separation of Ivabradine hydrochloride. The mobile phase was pumped through the column with a flow rate of 1 ml/min. The column was at an ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of drug solution. 20 $\mu$ l of standard and sample solutions were injected in to the chromatographic system and the area for the galantamine peak was measured. The detection of drug was monitored at 265 nm. The run time was set as 6 min. Under these optimized chromatographic conditions, the retention time for the drug was 4.36 min. The typical chromatograms of both standard and sample showing the separation of drug are given in figure 2 and 3. From the peak of Ivabradine the % assay was calculated.

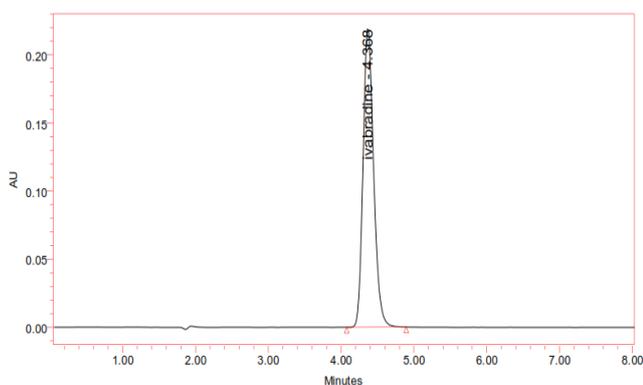


Fig. 2: Typical chromatogram of ivabradine hydrochloride from standard solution

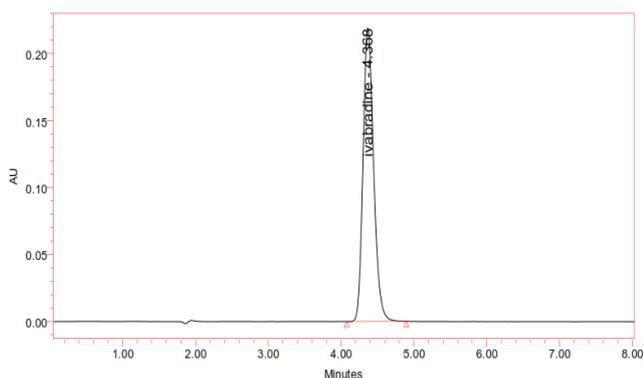


Fig. 3: Typical chromatogram of ivabradine hydrochloride from sample solution

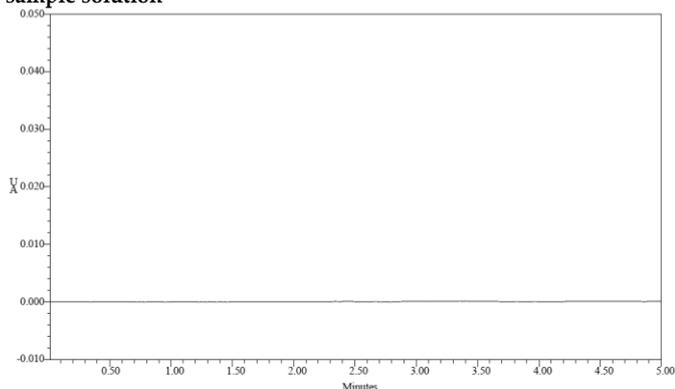


Fig. 4: Chromatogram of blank preparation

Table 1: System suitability results for ivabradine hydrochloride

Injection No	RT (min)	Area	Tailing	Plate count
1	4.378	2216085	1.2	4226
2	4.372	2219524	1.2	4143
3	4.368	2217869	1.2	4398
4	4.361	2233932	1.2	4386
5	4.356	2203923	1.2	4425
Mean	4.367	2218266		
Std dev	0.017	16923		
%RSD	0.38	0.76		

## RESULTS AND DISCUSSION

### Method Optimization

Different chromatographic conditions were tested to develop a liquid chromatographic method for the assay. The HPLC method was optimized through the evaluation of several solvent mixtures and columns. Mobile phase of methanol and phosphate buffer pH 6.5 in the ratio of 65:35% v/v on thermosil C<sub>18</sub>, 150 × 4.5

mm, and 5µm resulted sharp, well defined peak and good resolution and low retention time was about 4.36 min at the flow rate of 1 ml/min.

### Study of retention time

A standard dilution of pure drug having 100µg/ml of Ivabradine hydrochloride was prepared in diluent and loaded injection port of instrument fitted with 20µl fixed loop. The solution was injected and chromatogram was recorded. The mean retention time of Ivabradine was found to be 4.36 min. The respective chromatogram of Ivabradine is shown in figure 2 and 3.

### System suitability

After equilibration of column with mobile phase, five replicate injections of 20µl solution of standard solution was injected through the auto injector and the chromatograms were recorded. The system suitability parameters were noted and results are shown in Table 1.

### Method validation

The validation of analytical method verifies that the characteristics of the method if they satisfy the requirements of the method. Proposed method was validated according to ICH guidelines for specificity, linearity, accuracy, and precision, limit of detection and quantification and robustness. Specificity was carried out in which no interference of excipients was observed at retention time of the analytical peak. Calibration curve was constructed by plotting concentration Vs plot area. It showed that there was a good linear relationship in the concentration range of 30-150µg/ml with > 0.99 as the value of correlation co-efficient. The accuracy of the method was studied by analyzing the drug solutions at 50%, 100% and 150% concentration level. The mean percentage recovery was found to 100.5%. For precision the sample solution at working concentration was analyzed in replicate as per the method. The percentage relative standard deviation was found to be less than 1%. The limit of detection and limit of quantification of ivabradine were 2.97 and 9.92 respectively. The robustness of the method was determined to evaluate small but deliberate modifications in the chromatographic conditions of the original method. Robustness was determined by changing the flow rate of mobile phase to 0.7, 0.8 and 0.9 and by changing the % of methanol in mobile phase to 10% less and 10% more.

### Specificity

The specificity study was performed by injecting the blank. The chromatograms of blank, standard and sample (prepared from formulation) solutions were compared and it was found that there was no interference due to excipients from capsule formulation at retention time of ivabradine peak and moreover showed good correlation between the retention times of standard and sample.

### Linearity

Linearity of response (peak area) for ivabradine in relation to concentration was determined in a concentration ranging from 30-150µg/ml. Each

concentration level was injected twice in to the HPLC system. The linearity of calibration curve was validated by the value of correlation coefficient 0.992 of regression line and good correlation was obtained between the peak area and concentration as shown in figure 5 and the results are shown in the Table 2.

**Table 2: Linearity results for ivabradine hydrochloride**

Ivabradine (ppm)	Rt	Area
30	5.745	201932
60	6.019	338071
90	5.891	597859
120	6.030	740654
150	6.177	950396
Slope		6331.7
Intercept		4070.9
Co efficient of correlation (R <sup>2</sup> )		0.992

### Accuracy

The accuracy was evaluated by standard addition method by preparing concentration levels of 50%, 100% and 150% with respect to target assay concentration and each level was injected thrice into the chromatographic system and the mean percent recoveries for ivabradine hydrochloride were calculated and results are shown in Table 3.

**Table 3: Accuracy data for ivabradine hydrochloride**

Accuracy level (%)	Amount added (in mg)	Amount found (in mg)	% Recovery	Mean recovery
50	5	5.02	100.4%	
50	5	5.04	100.8%	100.7%
50	5	5.05	101.0%	
100	10	10.01	100.1%	
100	10	10.02	100.2%	100.4%
100	10	10.05	100.5%	
150	15	15.10	100.6%	
150	15	15.15	101.0%	100.8%
150	15	15.14	100.9%	

**Table 4: Repeatability data for ivabradine hydrochloride**

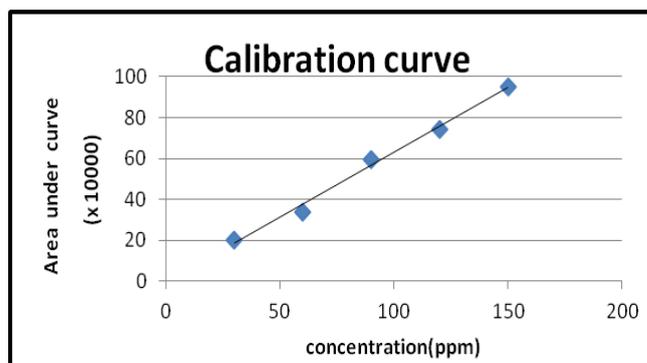
S. No	Peak name	RT	Area	Height	USP plate count	USP tailing
1	Ivabradine	4.354	2296680	223450	4346	1.2
2	Ivabradine	4.353	2233560	222927	4319	1.2
3	Ivabradine	4.348	2232042	223257	4384	1.2
4	Ivabradine	4.346	2231039	221910	4291	1.2
5	Ivabradine	4.344	2237718	222081	4244	1.2
6	Ivabradine	4.337	2225541	221980	4318	1.2
	Mean	4.3	222470			
	Std. Dev	0.0	10157			
	%RSD	0.1	0.5			

**Table 5: Intermediate precision data for ivabradine hydrochloride**

S. No	Peak name	RT	Area	Height	USP plate count	USP tailing
1	Ivabradine	4.354	2246680	223450	4346	1.2
2	Ivabradine	4.353	2233560	222927	4319	1.2
3	Ivabradine	4.348	2232042	223257	4384	1.2
4	Ivabradine	4.346	2231039	221910	4291	1.2
5	Ivabradine	4.344	2237718	222081	4244	1.2
6	Ivabradine	4.337	2225541	221980	4318	1.2
	Mean	4.3	2234430			
	Std. Dev	0.0	7181.2			
	%RSD	0.1	0.3			

### Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and quantification were determined based on the signal to noise ratio. The



**Fig. 5: calibration curve of ivabradine hydrochloride**

### Precision

The precision of the method was demonstrated by repeatability and intermediate precision studies. It was determined by testing the same batch of capsules according to description of analytical method by different analysts on different days using different HPLC and column of same make but different lot number. For both the studies six repeated injections of standard and sample solutions were made and % RSD for response factor of ivabradine was found to be less than 1% and results are shown in Table 4 and 5.

signal to noise ratio for LOD is 3:1 and LOQ is 10:1. The limit of detection and quantification were evaluated by applying statistical calculation to the linearity results.

The limit of detection (LOD) expressed as: **LOD=3.3  $\sigma$ /S** Where:

$\sigma$  = Standard deviation of response

S= Slope of the regression line

The limit of quantification (LOQ) expressed as:

**LOQ=10.0  $\sigma$ /S** Where:

$\sigma$  = Standard deviation of response

S = Slope of the regression line

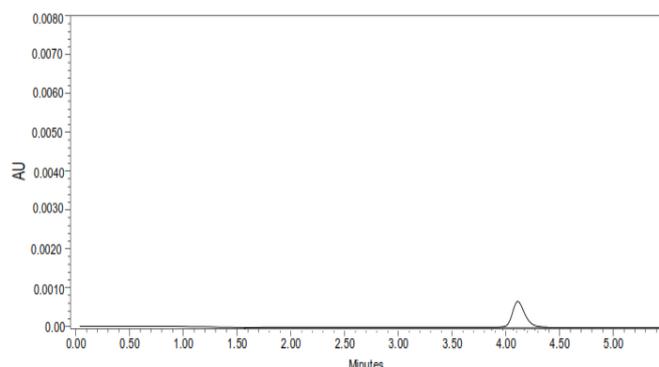


Fig. 6: Chromatogram of limit of detection

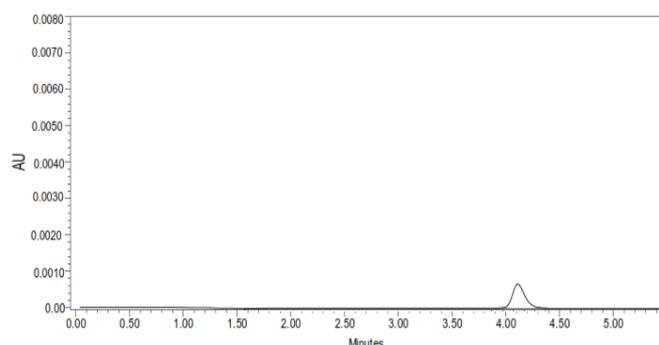


Fig. 7: chromatogram of limit of quantification

Table 6: Below table shows LOD and LOQ data for ivabradine hydrochloride

Standard deviation ( $\sigma$ )	Slope (s)	LOD ( $\mu\text{g}$ )	LOQ ( $\mu\text{g}$ )
371827.90	563365963	2.97	9.92

Table 7: Below table shows robustness data for ivabradine hydrochloride

Parameters	RT (min)	USP Plate Count	USP Tailing
Normal	4.3	4146	1.2
Flow rate 0.9ml/min	4.5	4352	1.2
Flow rate 1.1ml/min	3.9	4024	1.2
Mobile phase 65:35v/v	4.3	4331	1.2
Mobile phase 55:45v/v	4.1	4221	1.2

### Robustness

The robustness study of developed analytical method was determined by deliberate modification in flow rate and composition of mobile phase. The system suitability study was analyzed under these changed experimental conditions. The result shows that there is no significant change in  $R_t$  between original method and changes made to the method.

The developed method was found to be selective, accurate and reproducible for the analysis of ivabradine hydrochloride in capsules. The reagents utilized in the proposed method are cheaper, readily available and the procedures do not involve any tedious sample preparations. The method is unaffected by slight changes in chromatographic conditions such as flow rate and mobile phase ratio. The developed RP-HPLC method can be successfully employed for routine quality control analysis of ivabradine hydrochloride in pharmaceutical dosage forms.

### ACKNOWLEDGEMENTS

This work was supported by St. Mary's college of pharmacy. The authors would like to thank St Mary's college of pharmacy, for providing research facilities.

### REFERENCES

- De Silva R, Fox KM. Angina: ivabradine for treatment of stable angina pectoris. *Nature Reviews Cardiology*. 2009 May 1; 6(5):329-30.
- Postea O, Biel M. Exploring HCN channels as novel drug targets. *Nature reviews. Drug discovery*. 2011 Dec 1; 10(12):903.
- DiFrancesco D. Cardiac pacemaker/f current and its inhibition by heart rate-reducing agents. *Current medical research and opinion*. 2005 Jul 1; 21(7):1115-22.
- Seerapu S, Srinivasan BP. Development and validation of RP-HPLC method for the estimation of ivabradine hydrochloride in tablets. *Indian journal of pharmaceutical sciences*. 2010 Sep; 72(5):667.
- Rahman R, Asaduzzaman MD, Ashraf SM. Development and validation of RP-HPLC method for analysis of Ivabradine Hydrochloride in tablet dosage forms. *Res J Pharm Biol Chem Sci*. 2012; 3:1034-43.
- Motisariya MH, Patel KG, Shah PA. Validated stability-indicating high performance thin layer chromatographic method for determination of Ivabradine hydrochloride in bulk and marketed formulation: an application to kinetic study. *Bulletin of Faculty of Pharmacy, Cairo University*. 2013 Dec 31; 51(2):233-41.
- Maheshwari S, Khandhar AP, Jain A. Quantitative determination and validation of ivabradine HCl by stability indicating RP-HPLC method and spectrophotometric method in solid dosage form. *Eurasian Journal of Analytical Chemistry*. 2010 Mar 9; 5(1):53-62.
- Selva Kumar P, Rajagopal K. Development and Validation of Stability Indicating HPLC method for estimation of related substances in ivabradine hydrochloride in solid oral dosage form. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4(9): 1841-1853.
- Damle MC, Bagwe RA. Development and validation of stability-indicating HPTLC method for ivabradine HCl. *Pharm Sci Monitor*. 2015 Jan 1; 6(1):141-52.
- Patel H, Jivani N. Development of Validated RP-HPLC method for simultaneous estimation of carvedilol and ivabradine. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4(5): 630-639.
- Mostafa NM, Fayez YM, Farid JF, Abd El-Alim AE. Stability Indicating Spectrophotometric Methods for Determination of Ivabradine Hydrochloride in the Presence of Its Degradation Product. *Analytical Chemistry Letters*. 2017 Mar 4; 7(2):280-94.
- Klippert P, Jeannot JP, Polvé S, Lefèvre C, Merdjan H. Determination of ivabradine and its N-demethylated metabolite in human plasma and urine, and in rat and dog plasma by a validated high-performance liquid chromatographic method with fluorescence detection.

- Journal of Chromatography B: Biomedical Sciences and Applications. 1998 Nov 20; 719(1):125-33.
13. Lu C, Jia Y, Yang J, Jin X, Song Y, Liu W, Ding Y, Sun X, Wen A. Simultaneous determination of ivabradine and N-desmethylyvabradine in human plasma and urine using a LC-MS/MS method: application to a pharmacokinetic study. *Acta Pharmaceutica Sinica B*. 2012 Apr 30; 2(2):205-12.
  14. Zoerner AA, Schroeder C, Kayacelebi AA, Suchy MT, Gutzki FM, Stichtenoth DO, Tank J, Jordan J, Tsikas D. A validated, rapid UPLC-MS/MS method for simultaneous ivabradine, reboxetine, and metoprolol analysis in human plasma and its application to clinical trial samples. *Journal of Chromatography B*. 2013 May 15; 927:105-11.
  15. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov; 1.

**HOW TO CITE THIS ARTICLE:** Muzaffar-ur- Rehman M, Nagamallika G. Validated RP-HPLC Method for the Determination of Ivabradine Hydrochloride in Pharmaceutical Formulation. *Int. J. Pharm. Sci. Drug Res.* 2017; 9(5): 228-233. DOI: [10.25004/IJPSDR.2017.090505](https://doi.org/10.25004/IJPSDR.2017.090505)