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HPLC METHOD DEVELOPMENT FOR IVABRADINE HYDROCHLORIDE USING QUALITY BY DESIGN APPROACH

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ABSTRACT

Chromatographic and spectrophotometric methods were developed by Quality by Design approach according to ICH Q8 (R2) rules for assessment of Ivabradine Hydrochloride. The basic boundaries were controlled by utilizing head part examination just as by perception. In this examination, the Quality by Design approach was utilized to build up a superior fluid chromatography technique that could be applied for the assessment of Ivabradine HCL. The technique created utilizing C18 segment with portable stage containing 0.1 M Potassium Dihydrogen Orthophosphate and Acetonitrile in the extent 45:55 at a stream pace of 1.2 ml/min and discovery was completed utilizing a PDA indicator at 285 nm. Proposed techniques can be utilized for routine investigation of Ivabradine hydrochloride in tablet measurements structure as they were discovered to be powerful and explicit.

INTRODUCTION

Ivabradine Hydrochloride is the hydrochloride salt type of Ivabradine, an orally bioavailable, hyperpolarization-initiated, cyclic nucleotide-gated (HCN) channel blocker, with negative chronotropic action. Upon organization, Ivabradine specifically ties to the intracellular segment of the HCN channel pore and squares HCN directs in the pacemaker cells inside the sinoatrial (SA) hub. This represses the If pacemaker particle current, forestalls the internal stream and intracellular gathering of decidedly charged particles, lessens pacemaker action and eases back diastolic depolarization. This declines pulse, lessens myocardial oxygen interest and permits more opportunity for blood to stream to the myocardium without influencing heart contractility. HCN channels, blended sodium (Na+) and potassium (K+) channels that convey the internal if current, assume a critical part in the guideline of pacemaker Terminating rate in the SA hub. The If pacemaker current, the internal progression of decidedly charged Na+-K+ particles, starts the unconstrained diastolic depolarization stage and adjusting pulse. Ivabradine Hydrochloride is Chemically 3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo [4.2.0] octa-1,3,5-trienyl]methyl-methyl amino] propyl] -7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one;hydrochloride.

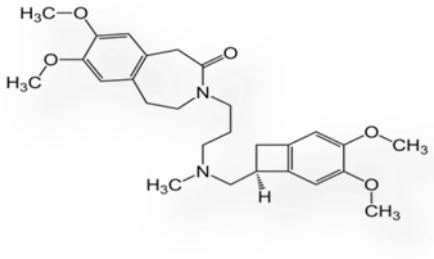


Figure: 1 Structure of Ivabradine Hydrochloride

MATERIALS AND METHODS:

Chemicals: Ivabradine HCL received as gift sample from Sun Pharma, Vadodara, Gujarat

and other chemicals used were HPLC grade (Merck).

Chromatographic Conditions: The HPLC framework utilized was Agilent 1260 Infinity with Autosampler (AS-4050) and a PDA locator and implicit degasser. The framework utilized Open Lab EZ Chrome programming. After different preliminaries, C18 column was chosen with a portable stage made out of 0.1 M Potassium Dihydrogen Orthophosphate and Acetonitrile. The stream rate was kept up at 1.2 ml/min and a finder set to frequency 294 nm. Autosampler utilized had variable circle volume 0-100 µl, and in this strategy, 20 µl was infused. The framework had a segment stove making it conceivable to program section temperatures during the run. After a preliminary at different temperatures, it was chosen to set the segment temperature at 40 °C all through the technique.

Preparation of stock solution:

Accurately weighed 10 mg of Ivabradine HCL was transferred to 10 ml volumetric flask containing little amount of 0.1 M Potassium Dihydrogen Orthophosphate and Acetonitrile in the extent 45:55. The volume was made up to the mark using same mixture of mobile phase to get 1000 ppm concentration.

Preparation of working solution:

From the stock solution, withdraw 1 ml and transfer it to volumetric flask and dilute it with the mobile phase upto 10 ml (100 ppm). The resulting solution is sonicated for 10 min.

Method Development:

Selection and Preparation of Mobile Phase: Mobile phases containing methanol, water, acetonitrile, and cradles at various pH were attempted in various extents and at various stream rates. Good pinnacles were acquired at a stream pace of 1.2ml/min with a versatile stage comprised of 45 sections Potassium Dihydrogen Orthophosphate and 55 pieces of Acetonitrile. The two parts of the portable stage were separated through 0.45µm film channels by utilization of vacuum and sonicated for 15 min prior to bringing into the framework.

Preparation of Standard Stock Solutions:

The standard solutions of the drugs were prepared in Acetonitrile. A quantity of 10 mg of each drug was weighed and dissolved in Acetonitrile in 10 ml volumetric flasks, to give standard stock solutions of 1000 µg/mL of each drug. The standard stock solutions were further diluted with Acetonitrile to obtain required concentrations of each drug. All solutions, including stock solution, were freshly prepared each day.

Preparation of Calibration Curve: Volumes of standard stock arrangements of each medication were moved to a 10 mL volumetric flagon and weakened sufficient with methanol. Aliquots were taken in such a manner to acquire last fixations in the scope of 10-150 µg/mL for each medication. Calibration curve were plotted for each medication by plotting top regions recorded for every fixation on the y-pivot and the centralization of the medication on the x-hub. The coefficient of assurance (R2) was determined for the alignment bend of each medication.

Experimental Design

Factorial Design

A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert®

Method

Validation: The technique created was approved according to ICH rules by assessing boundaries, for example, exactness, accuracy, linearity, heartiness, and roughness, recognition, and measurement limits. The outcomes were assessed thinking about satisfactory cutoff points as under 2% for Relative Standard Deviation (RSD).

Precision: The accuracy of the created technique was affirmed for every one of the medications. The pinnacle territories recorded by genuine investigation of six imitate infusions of a standard centralization of each medication. The exactness of the technique was additionally checked as far as the intra-and between day variety in the pinnacle zones by computing the RSD.

Accuracy: The accuracy of the method was tested for each of the drugs by spiking a known concentration of each drug at three

different concentration levels, namely 50%, 100%, and 150%, and then comparing the difference between the expected/theoretical value and the concentration determined by the method.

Linearity: A stock solution of 1000 µg/mL in methanol was prepared for Ivabradine HCL. From this stock, working standard solutions were prepared for each of the drugs, in the range of 10 to 150 µg/mL and injected into the HPLC system. It was proved that each drug shows linearity in the range of 10–150 µg/mL. The calibration graph (obtained by plotting peak areas of the drug under consideration versus its concentration) was generated by replicate analysis at all concentration levels, and the linearity of the relationship was established using Microsoft Excel® program.

RESULTS AND DISCUSSION:

Method Development:

Chromatographic Separation: After a number of trials, chromatographic conditions were optimized and selected based on System Suitability parameters. The optimized chromatographic conditions are reported in **Table 1**.

Representative HPLC Chromatogram is shown in **Fig. 2**.

System Suitability parameters for each drug were checked and are tabulated in **Table 2**.

Calibration Curve: The correlation coefficients (R^2) for each of Ivabradine HCL under consideration and also the linearity equations are displayed in **Table 3**.

Method Validation: The method was validated

1. Accuracy: The percentage recovery of Ivabradine HCL

2. Linearity: The linearity response was determined by analyzing independent levels of Calibration curve in the range of 10-150 µg/ml for Ivabradine HCL

3. Precision: The value of Ivabradine HCL was found within Limit, which indicates that the developed method is precise.

4. Specificity: The value of Ivabradine HCL was found within Limit, which indicates that the developed method is specific.

Table 1: Optimized Chromatographic Conditions

S.No.	Parameters	Values
1	Stationary phase	C18
2	Mobile phase	0.1 M Potassium Dihydrogen Orthophosphate and Acetonitrile
3	Flow rate (mL/min)	1.2 ml/min
4	Run time (min)	3 min
5	Column Temperature	40 °C
6	Injection Volume (µl)	20 µl
7	Detection Wavelength (nm)	285 nm

Table 2: Coded Values for Independent Variables

Name of Factor	Code Values	Levels		
		Small	Medium	High
Mobile Phase Ratio	A	35:65	45:55	55:45
Flow rate	B	1	1.1	1.2

Table 3: Different Batches with their Respective Composition

Batch Code	Mobile Phase Ratio	Flow rate
P1	35:65	1
P2	35:65	1.1
P3	35:65	1.2
P4	45:55	1
P5	45:55	1.1
P6	45:55	1.2
P7	55:45	1
P8	55:45	1.1
P9	55:45	1.2

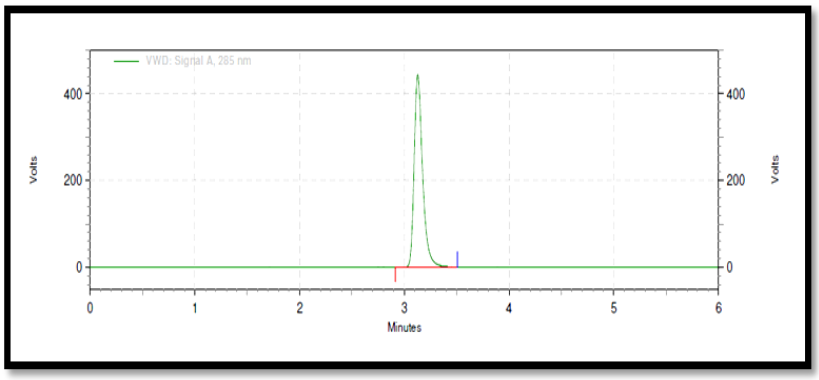


Figure 2: Chromatogram of Ivabradine HCL

Table 4: System Suitability Parameters

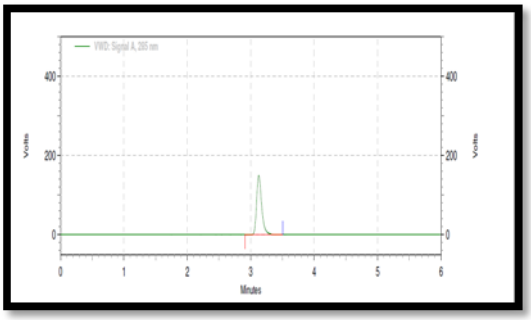
S.No.	Parameters	Acceptance Criteria	Ivabradine HCL
1	Theoretical Plates	>1000	**7879**
2	Tailing factor	<2	**0.768**
3	RSD of area	<2%	**0.167**
4	RSD of Ret.Time	<1%	**0.171**

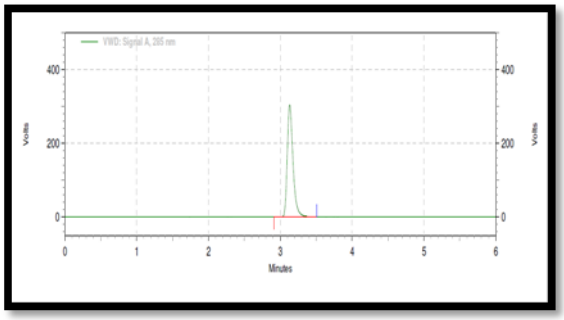
Table 5: Correlation Coefficients and Linearity Equations

Sr. No.	Drug	Regression Value	Equation
1	Ivabradine HCL	1	y = 1.000x + 0.002

Table 6: Result of Accuracy (%Recovery)

Sr. No.	Assay Level	% Recovery
1	50	99.3
2	100	100.9
3	150	99.5





A **B**

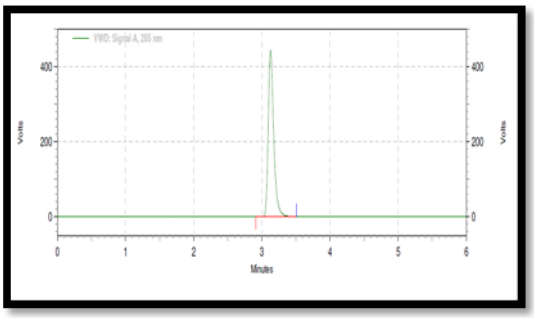


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Figure3: Chromatograms at Assay Level 50%, 100% and 150%

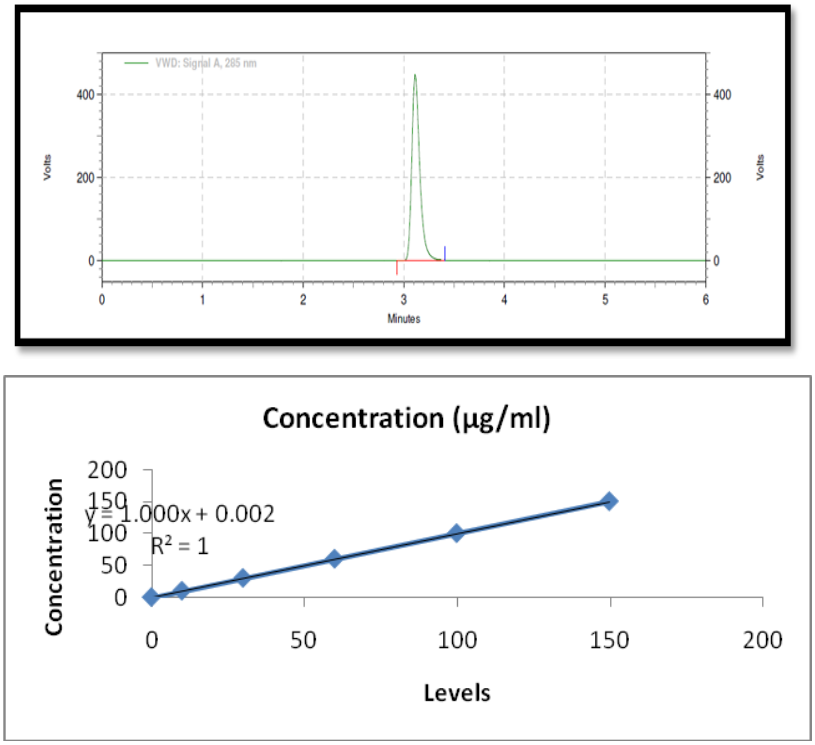

Figure 4: Chromatograms for linearity

Table 7: Result of Precision (%Recovery)

Sr. No.	Evaluation Parameter	Results	Acceptance Criteria
1	% Assay values obtained by six test solutions (Average)	100.1	NLT 98% and NMT 102 %
2	% RSD for Assay values obtained by six test solutions	1.2	NMT 2.0 %

Table 8: Result of Specificity

Sr. No.	Results	Acceptance Criteria
1	Retention time of Ivabradine peak in test solution is comparable to that in standard solution.	Retention time of Ivabradine peak in test solution should be comparable to that in standard solution.
2	peak purity of standard and test solution is within acceptance criteria	NLT 950

CONCLUSION:

QbD approach for logical strategy improvement that comprises of (i) building up a full comprehension of the expected reason, (ii) creating prescient arrangements, (iii) planning a significant framework reasonableness arrangement that assists with distinguishing disappointment modes, and (iv) following plan of tests way to deal with the technique advancement has been introduced. These ideas were effectively applied to the turn of events and Enhancement HPLC technique for drug. A full comprehension of the item was assembled to create technique execution assumptions remembering basic sets for the chromatographic partition and furthermore in creating prescient test arrangements. The created framework reasonableness arrangement was significant since it helped in guaranteeing chromatographic partition of tops in examples from steadiness considers. The QbD approach had been effectively

used to create HPLC strategy discovered to be simple, quick, sensitive, economical, reliable, and precise.

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