Research Article

Quantitative determination and validation of novel derivative spectrophotometric method for estimation of Ivabradine hydrochloride in bulk and marketed formulation

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Abstract

Objective: A simple, rapid, highly sensitive, selective, specific, robust UV spectrophotometric method has been developed and validated for the estimation of Ivabradine HCL in bulk and pharmaceutical dosage form. **Material and Methods:** Spectroscopic determination was done by zero order and first order derivative spectroscopic method by using water an ideal solvent and λ max was found to be 286nm. Beer's Lambert's Law was obeyed within the range of 10-30µg/ml for Zero, First order method. **Results:** The method was validated according to ICH for the validation parameters accuracy, precision, LOD, LOQ, specificity. The correlation coefficient (r^2) of method was found to be 0.9997 and 0.998 for zero and first order method respectively. The intra-day and inter-day precision and accuracy values for both the methods were found to be less than 2% RSD. **Conclusion:** The Proposed UV spectroscopic methods have been successfully applied to the commercial tablets without any interference of excipients. The method can be used for the routine analysis in industry of Ivabradine HCL in bulk and tablet dosage forms.

Keywords: Ivabradine hydrochloride, UV spectroscopy, Derivative Spectroscopy, validation, first order derivative

Introduction

The chemically Ivabradine hydrochloride is 3-(3-{[((7S)-3,4-Dimethoxybicyclo [4,2,0] octa-1,2,3-trien-7-yl) methyl] methyl amino} propyl)- 1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride (Figure1) corresponding to the molecular formula ($C_{27}H_{36}N_2O_5$.HCL). Ivabradine HCL has a relative molecular mass of 505.05 g/mole (Martindale, 2007).

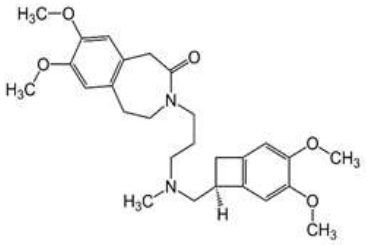

Figure 1. Chemical structure of Ivabradine Hydrochloride

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Recently, ivabradine was approved by the U. S. Food and Drug Administration (USFDA) as a heart-rate-lowering agent in 2015. Ivabradine is the first drug with a novel mechanism of action acting by specifically and selectively inhibiting the cardiac pacemaker current (If), a mixed sodium-potassium inward current that controls the spontaneous diastolic depolarization in the sinoatrial (SA) node and hence regulates the heart rate (Sabharwal and Kukin, 2015). The molecular channel of Ivabradine HCl belongs to the HCN family. If ion current flow is disrupts by the inhibition of this channel, thereby prolonging diastolic depolarization, slowing firing in the SA node, and eventually reducing the heart rate (Psotka and Teerlink, 2016). The Cardiac effects of Ivabradine are specific to the SA node, and the drug has no effect on blood pressure, intracardiac conduction, ventricular repolarization, myocardial contractility (Tse and Mazzola, 2015).

The sinoatrial node is unique in that its cells have an innate ability to generate a cyclical change in their resting membrane potential, which drives it toward the threshold needed for spontaneous depolarization. This depolarization, in turn, generates repetitive, spontaneous action potentials

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accounting for its automaticity. This depolarization is initiated by the opening of specific ion channels that conduct a slow, inward-depolarizing mixed sodium-potassium current, referred to as the pacemaker or "funny" current (If). If is generated via a nonselective, hyperpolarization-activated cyclic nucleotide–gated transmembrane channel. Ivabradine blocks the intracellular aspect of this transmembrane channel by inhibiting cation movement with a high degree of selectivity, leading to a reduction in the slope of the diastolic depolarization of the pacemaker action potential, thereby slowing the heart rate. Ivabradine blocks the channel in its open state, creating a particularly favorable attribute, use dependence (i.e., it becomes more potent at faster heart rates). Ivabradine causes a dose-dependent reduction in heart rate and, as a consequence of its specific mechanism of action; it is able to do so without affecting cardiac inotropy or systemic vascular resistance (Jedličková, 2014; Koruth et al., 2017).

The literature survey reveals that numerous methods for determinations of Ivabradine HCL in single (Islam et al., 2012; Srinivasan et al., 2010) in pharmaceutical dosage forms, spectrophotometric methods in combination with other drugs (Patil, 2016), HPTLC (Damle et al., 2015; Patel et al., 2013), stability indicating spectrophotometric (Mostafa et al., 2017; Bhosale et al., 2016) and HPLC methods in combination with other drug including HPLC (Patel et al, 2015).

In this study we described very simple, sensitive, novel spectrophotometric methods. The zero order and first order derivative spectroscopic methods are not available for estimation of Ivabradine HCl in single component and for simple analysis on bulk and tablet dosage form. These methods show very simple and accurate approach for the analysis of Ivabradine HCL without need of sophisticated instruments, expensive solvents or a large number of samples.

Materials and methods

Materials

Pure sample of Ivabradine HCL was kindly supplied as a gift sample by Lupin Pharmaceuticals (Aurangabad, Maharashtra) India. All solvents and chemicals were of analytical grade. Marketed Tablet dosage form used in this research work was IVABRAD 5mg (Lupin Laboratories) acquired from local market.

Instruments

Spectrophotometric measurements were carried out on Shimadzu UV 1800 double beam spectrophotometer. Infrared spectroscopic study was done on FTIR (Bruker, Japan).

Preparation of standard stock solution

Standard stock solution of Ivabradine Hydrochloride was prepared by accurately weighing 100 mg of Ivabradine Hydrochloride to 100 ml volumetric flask with specific volume of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

Selection of analytical wavelength for zero order derivative method

0.1mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was than scanned in UV range between 200-400nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as water. The absorption maxima were found to be 286nm and it is shown in figure 2.

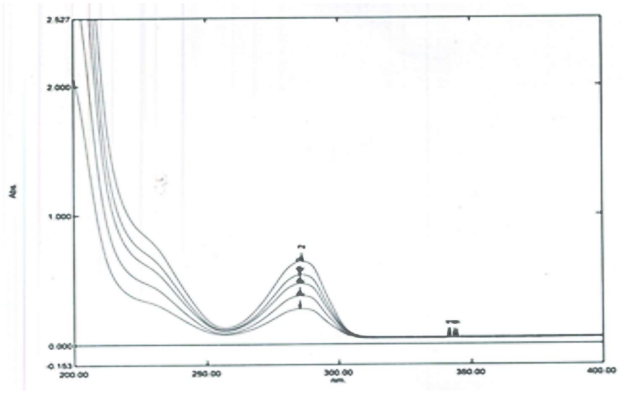

Figure 2. Zero Order Derivative Spectra of Ivabradine Hydrochloride

Selection of analytical wavelength for first order derivative method

The 0.1 mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was scanned in the wavelength range of 200-400 nm using UV spectrophotometer. The conversion of normal spectrum into first order derivative spectrum was done. The spectrum shows the sharp peak and maximum absorbance at 276nm. The λ_{max} 276 was selected for the first order derivative analysis and it is shown in figure 3.

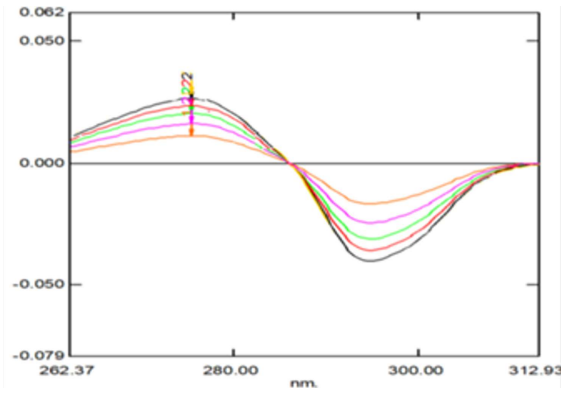

Figure 3. First Order Derivative Spectra of Ivabradine Hydrochloride

Preparation of calibration curve for zero and first order derivative methods

Aliquots portion 0.1, 0.15, 0.2, 0.25, 0.3 ml was pipetted out from the standard stock solution and transferred to series of 10 mL volumetric flask and volume was made with water to get the concentration range from 10-30µg/ml. The absorbance was measured three times for each concentration. Absorbance of each solution was measured against water as blank at 286 nm and 276nm for zero order and first order methods respectively.

Analysis of tablet formulation

Twenty tablets were weighed and finely powdered. Equivalent to 10 mg of Ivabradine Hydrochloride was weighed and transferred to a 100 ml volumetric flask containing with specific amount of water and sonicated for 20 minutes. The solution was filtered through 0.45µm Whatmann filter and volume was made up to mark with water and mixed to get 100µg/ml. An aliquot of tablet stock solution 1 ml was transferred to 10 ml volumetric flask and volume was made up to mark with water to get concentration of 10µg/ml of Ivabradine Hydrochloride. Recovery study of tablet formulation is shown in table 1.

Table 1. Data for tablet analysis (Label Claim)

Parameters	Zero Order	First Order
Tablet	Ivabrad (5mg)	Ivabrad (5mg)
Mean*(n=6)	100.33	100.92
SD	0.1631	0.0623
% RSD	0.1625	0.0617

Method validation

According to ICH Q2 (R1) guidelines the developed methods were validated to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness.

Statistical Analysis

Statistically the terms like SD and %RSD calculated in presented study. The standard values are mentioned in ± SD.

$$\% \, \mathbf{RSD} = \frac{\mathbf{SD}}{\mathbf{Mean}} \times \mathbf{100}$$

$$\mathbf{SD} = \frac{\sqrt{\sum(\mathbf{X} - \overline{\mathbf{X}})}}{\mathbf{N} - \mathbf{1}}$$

Where,

SD: Standard Deviation

%RSD: Relative standard deviation

N: Number of data values in data set

X: Each of values of the data set

Table 2. Data for accuracy (Recovery) Study

Zero Order		First Order	
Level of addition	**% recovery (n=3)**	**Level of addition**	**% recovery (n=3)**
80%	99.02 ± 0.12	80%	100.48 ± 0.33
100%	100.07 ± 0.17	100%	100.26 ± 0.16
120%	99.84 ± 0.27	120%	101.31 ± 0.28

Results and discussion

Linearity

The linearity of proposed derivative methods was evaluated by plotting the absorbance against concentrations of standard drug solutions. Stock solutions was consequently diluted with water to get 10, 15, 20, 25, 30µg/ml. The λmax for first order derivative was obtained by converting the normal spectrum of zero order spectrum to first order spectrum. The correlation coefficient was found to be 0.9997 and 0.9983 for zero order and first order derivative method.

Precision

The Precision study of analytical method validation express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of day precision of proposed methods was evaluated by analysing the three different independent concentrations i.e. 10, 15, 20 µg/ml in triplicate. These concentrations were evaluated for three consecutive days and the results are given in table 3 and table 4 for zero order and first order derivative methods.

Table 3. Data for Precision Study for Intra-Day and Inter-Day (Zero Order)

Conc	Intra-Day Precision		Inter-Day Precision	
(µg/ml)	**Amount Found(n=3)**	**%RSD**	**Amount Found(n=3)**	**%RSD**
10	9.96 ± 0.0007	0.32	9.98 ± 0.0007	0.33
15	14.93 ± 0.0012	0.38	14.98 ± 0.0012	0.39
20	20.11 ± 0.0005	0.12	19.96 ± 0.13	0.13

Table 4. Data for Precision Study for Intra-Day and Inter-Day (First Order)

Conc	Intra-Day Precision		Inter-Day Precision	
(µg/ml)	**Amount Found(n=3)**	**%RSD**	**Amount Found(n=3)**	**%RSD**
10	10 ± 0.00071	0.51	10.01 ± 0.0007	0.51
15	14.91 ± 0.0001	0.51	15.08 ± 0.00070	0.35
20	20.25±0.00070	0.26	20.21 ± 0.00217	0.82

Accuracy

The accuracy of an analytical procedure expresses the results obtained by that method to the true value. The accuracy of the developed method was determined on the basis of recovery studies. The recovery tests were performed by adding known quantity of pure standard drug into the solution of tablet powder. The sample was then spiked with standard at levels 80%, 100% and 120% of tests concentrations. The resulting spiked sample solutions were analysed in triplicate.

Limit of Detection (LOD) Limit of Quantitation (LOQ)

The LOD and LOQ were evaluated from the data obtained from calibration curve. The LOD and LOQ for zero order derivative method was found to be 0.117µg/ml and 0.355µg/ml respectively and for first order derivative was found to be 0.192µg/ml and 0.583µg/ml respectively.

Robustness

Robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness study of proposed method was evaluated by changing parameters like wavelength. In the proposed method the robustness study was studied by changing the wavelength (284 and 288) for zero order and (274 and 278) for first order derivative methods. The robustness testing for both the methods are given in table 5, which indicates that, no significant difference was observed in results. Thus, it's demonstrated that the methods are robust.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. Ruggedness study was performed to examine effect of non-procedure related factors such as instruments and analyst. Ruggedness study of Ivabradine Hydrochloride was carried out by using two different analyst under the similar operational and environmental conditions. The % RSD for zero order and first order derivative for change in analyst was found in limit (< 2%) which is shown in table 6.

Specificity

Specificity study is the ability to asses unequivocally the analyte in the presence of component which may be expected to be present. For the specificity study of proposed method the sample may be spiked with excipients or possible interfering components. Results of specificity study were found in analytical limits shown in table 7.

Conclusion

The developed analytical method for Ivabradine Hydrochloride by zero order derivative and first order derivative spectroscopy method was found to be linear, specific, rapid and precise. The solvent used in proposed methods is water is an economical and cheap, which indicates that the proposed methods are economic and cost effective. The %RSD for all validation parameters studied

Table 5. Data for Robustness Study

	Zero Order		First Order	
Parameters	**Wavelength (284nm)**	**Wavelength (288nm)**	**Wavelength (274nm)**	**Wavelength (278nm)**
Conc. (30µg/ml)	**30**	**30**	**30**	**30**
Mean(n=5)	0.619	0.615	0.382	0.379
SD	0.000612	0.00162	0.000935	0.002358
% RSD	0.0988	0.2634	0.2448	0.6221

Table 6. Data for Ruggedness Study

Parameters	**Zero Order**		**First Order**	
	Analyst I	**Analyst II**	**Analyst I**	**Analyst II**
Conc. (20µg/ml)	**20**	**20**	**20**	**20**
Mean(n=5)	0.417	0.419	0.266	0.266
SD	0.000866	0.000612	0.000619	0.00177
% RSD	0.2076	0.1460	0.2300	0.6650

Table 7. Data for Specificity Study

Level of Addition	**Tablet drug conc. (µg/ml)**	**Exipients Conc. (µg/ml)**	**Total conc. (µg/ml)**	**Zero Order**		**First Order**	
				Absorbance	**% RSD**	**Absorbance**	**%RSD**
80%	7	10	17	0.163	0.92	0.102	0.84
100%	10	10	20	0.217	0.73	0.136	0.51
120%	13	10	23	0.282	0.35	0.176	0.57

in proposed method was found to be less than 2%. The method is highly sensitive and entirely suitable for routine analysis of Ivabradine Hydrochloride in bulk and solid dosage forms.

Conflicts of interest

None

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