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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RAPID HPLC METHOD FOR ESTIMATION OF IVABRADINE HYDROCHLORIDE IN SOLID ORAL DOSAGE FORM

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ABSTRACT

Objective: A simple, rapid, accurate, linear, precise, specific, robust, rugged and stability indicating high performance liquid chromatography (HPLC) method has been developed for estimation of Ivabradine hydrochloride from solid oral dosage form.

Methods: The chromatographic separation was obtained using a mobile phase composition at a ratio of 50:50 (v/v) of 10 mM ammonium acetate buffer pH 6.0 and methanol on Phenomenex Kinetex C18 column (150 × 4.6 mm, 5 µm), ambient temperature with UV detection at 285 nm at a flow rate of 1.0 ml/minute. The retention was at 3.1 mins.

Results: The stability indicating capability of the method was proven by subjecting the drug to stress conditions as per ICH recommended test conditions such as acid and alkali hydrolysis, oxidation, photolysis, thermal and humidity degradation. The peak purity plots show that the Ivabradine hydrochloride peak is homogeneous and that there are no co eluting peaks indicating that the method is stability indicating and specific. The % RSD value was 0.48% for method precision. Ruggedness of the method is indicated by the overall RSD values of 0.52 % for Ivabradine hydrochloride. The linearity of response for Ivabradine hydrochloride was determined over the range 70.69 to 131.29 µg/ml with correlation coefficient of 0.99974. The mean accuracy of triplicate samples of three different levels was found at 99.03% with %RSD of 0.38%.

Conclusion: The analytical method validation data showed excellent results for precision, linearity, specificity, accuracy, ruggedness and robustness. The present method can be successfully used for routine quality control and stability studies. This method can also used for LCMS analysis as mobile phase is LCMS compatible.

Keywords: Force Degradation, Ivabradine hydrochloride, LCMS Compatible, RP- HPLC, Stability Indicating

INTRODUCTION

Ivabradine hydrochloride is 3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo[4.2.0]octa-1,3,5-trienyl] methyl-methylamino]propyl]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-onehydro chloride. Ivabradine hydrochloride is a therapeutic agent used for the symptomatic treatment of chronic stable angina pectoris in patients with normal sinus rhythm who cannot take beta blockers. Ivabradine hydrochloride is also indicated in combination therapy with beta-blockers in patients inadequately controlled by beta-blocker alone and whose heart rate exceeds 60 beats per minute. It found to be as effective as the beta-blocker atenolol and comparable with amlodipine in the management of chronic stable angina [1-5]. Through Literature survey shows that several RP-HPLC methods are reported for the determination of Ivabradine hydrochloride in urine, plasma and formulations with fluorimetric detection [6], mass spectrophotometric detection [7] and HPLC-UV detection [8, 9, 10] respectively. All this methods are having longer rum time varying from 12 to 15 min with retention time of 7-8 mins. In the present study main objective was to develop and validate rapid, simple, specific, stability indicating UV-HPLC method for estimation of Ivabradine hydrochloride in oral dosage form.

MATERIALS AND METHODS

Instrumentation

The HPLC consisted of Waters 2695 Separations Module (Alliance) with Waters 2487 Dual Wavelength Detector, Waters 2996 Photodiode Array Detector with Empower data processing software (Waters, Milford, MA, USA). Chromatography was performed on Phenomenex Kinetex C-18(150*4.6) mm, 5 µ column (Phenomenex, USA).

Chemicals and Reagents

API of Ivabradine hydrochloride with potency of 97.6 %(on as is basis) was procured from MDRL, Chennai, India. Methanol (HPLC grade), ammonium acetate (AR grade), acetic acid (AR grade), ammonia (AR grade), hydrochloric acid, sodium hydroxide hydrogen peroxide were purchased from Spectrochem (Mumbai, India). Water was purified using Siemens Ultra Pure Water System (Siemens Water Technologies, Lowell, Malaysia). The tablet dosage form contains 5mg Ivabradine hydrochloride (Ivabrad) from Lupin, Mumbai, India from local pharmacy.

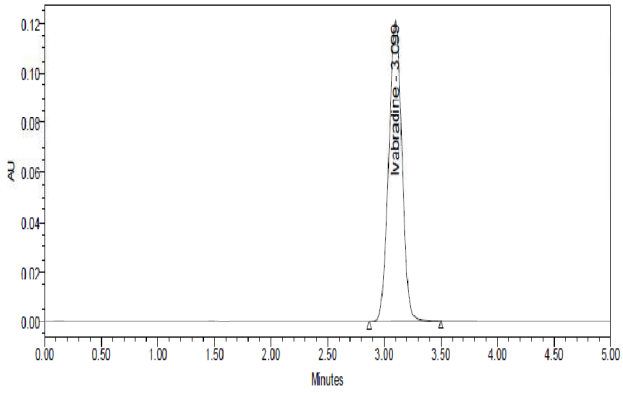

Fig. 1: Chromatogram of Ivabradine hydrochloride standard solution

Standard preparation

Standard stock solution was prepared by dissolving 54 mg of Ivabradine hydrochloride (Equivalent to 50 mg of Ivabradine) in water and made up the volume to 100 mL with water. Further above solution diluted 5 mL into 50 mL with water. The standard solution was filtered with 0.45µ Nylon filter (Millipore, Bedford, MA, USA) by discarding first few mL of the stock solution [Fig. 1].

Sample preparation

Average weight was determined for 20 tablets. The tablets were grinded it to fine powder by using mortar and pestle. Weighed equivalent to 10mg of Ivabradine and transferred the sample in 100 mL volumetric flask, added about 50 mL of water sonicated for 20 minute. Allowed the sample to attain room temperature and made up the volume to the mark with water. Allow the sample to settle. The sample solution were filtered with 0.45µ Nylon filter (Millipore, Bedford, MA, USA) by discarding first few mL of the stock solution.[Fig.2]

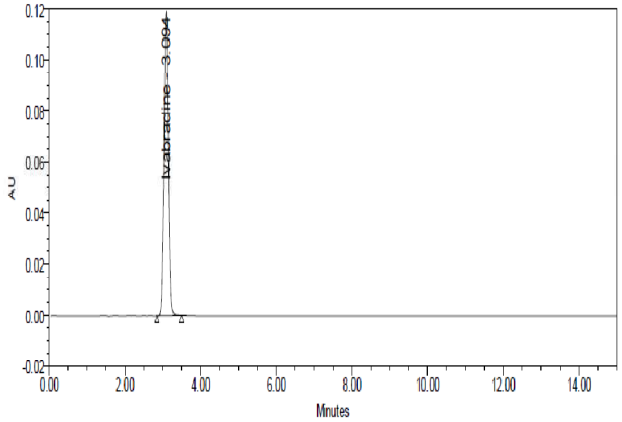

Fig. 2: Chromatogram of Ivabradine hydrochloride sample solution

Chromatographic condition

Chromatographic separation was achieved on a reverse phase column Phenomenex Kinetex C18 (150 × 4.6 mm, 5µm) at ambient temperature using a mobile phase consisting of a mixture of 10mM ammonium acetate solution pH 6.00 and Methanol in the ratio of 50:50(v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 285nm. The acetate buffer was filtered through a membrane filter (0.45µm). The injection volume used for assay and degradation studies was 10µl. The retention time of Ivabradine hydrochloride was 3.1 min.

Method Validation

The method validation study was carried out as per ICH guidelines [11, 12]. Below said parameters are evaluated as follows.

System Suitability

The system suitability test was performed to ensure that the HPLC method was suitable to the analysis intended. A standard solution containing 100µg/ml of Ivabradine hydrochloride was injected in five replicate injections. Chromatographic parameters including peak area, retention time, theoretical plates, tailing factors were measured and the relative standard deviation (RSD) was determined.

Precision

Six replicate injections of standard solutions were given into the HPLC system to establish injection repeatability or system precision. In Method precision study sample solution were analysed six times on the same day and %RSD was calculated.

Specificity

Specificity of method was determined by injecting blank, placebo and sample solution into HPLC system to check placebo interference at the retention time of Ivabradine hydrochloride and check co-elution for main peak.

Forced Degradation

To show the stability indicating capability of the method Ivabradine hydrochloride formulation sample were subjected to stress testing as per ICH recommended test conditions [12, 13]. The formulation was subjected to acid hydrolysis by using 1 N hydrochloric acid (10% / 75°C for 6 h), base hydrolysis by using 1 N sodium hydroxide (10% / 75°C for 6 h), oxidation by using 3% peroxide(10% /75°C for 6 h), thermal(105°c for 24 h), photolytic(1.2 million lux h & 200 W h / sq. m) and humidity(92%RH at 25°C for 48 h).

Linearity

Ivabradine hydrochloride solutions were prepared at concentrations of 70, 80, 90, 100, 110,120 and 130µg/ml.Standard plots were constructed and linearity was evaluated statistically by linear regression analysis that was calculated by least-squares regression.

Accuracy

Known amount of placebo was taken and spiked with Ivabradine hydrochloride API at three different levels (80%, 100% and 120%), each in triplicate. The results were expressed as the percentage of Ivabradine hydrochloride recovered from sample.

Robustness

Robustness of the method was checked by varying the instrumental conditions such as pH of buffer (± 0.2), flow rate (± 10 %), column oven temperature (+5°C), organic content in mobile phase (± 2 %) and wavelength of detection (± 5 nm). Sample solution was injected under each condition and assay of Ivabradine hydrochloride calculated. The results were evaluated for the mean, standard deviation and % RSD.

Ruggedness

Ruggedness of the method was verified by analysing samples by two different analysts using different instruments and columns on different days. The percent relative standard deviation (%RSD) of two sets of data was evaluated to show ruggedness of method.

Stability of the solution

A sample solution of Ivabradine hydrochloride was prepared and analysed initially and analysed at different time intervals by keeping the solution at room temperature.

RESULTS AND DISCUSSION

Method Development

The chromatographic conditions were optimized with a view to develop a stability indicating assay method with shorter run time. The chromatographic separation were tried using C-18 column chemistry with two different dimension (150 x 4.6 mm, 5 µm and 75 x 4.6 mm, 2.7 µm) and variable mobile phase composition, pH of Buffer and flow rate. The experimental studies showed that the column, Phenomenex Kinetex C18 (150 × 4.6 mm, 5µm) was most suitable, since it produced best chromatographic performance and acceptable peak shape including good plate count and peak tailing with shorter run time. The Chromatographic conditions finally comprised of a mobile phase composition at a ratio of 50:50 (v/v) of 10 mM ammonium acetate buffer pH 6.0 and methanol on Phenomenex Kinetex C18 column (150 × 4.6 mm, 5 µm), ambient temperature with UV detection at 285 nm at a flow rate of 1.0 ml/minute. The retention was at 3.1 mins.

Method Validation

System suitability

Standard solution was injected on different days during the validation studies. Using the system suitability software, the Column

efficiency and USP tailing for Ivabradine hydrochloride peak was calculated. Also, % RSD for replicate injections of Ivabradine hydrochloride peak was calculated and it is within 2% indicating the suitability of the method [Table 1].

Precision

The relative standard deviation for six replicate injections of the standard solution for analyte lie well within the limits (% RSD ≤ 2.0), showing the injection repeatability or system precision of the method.

The values of the percent relative standard deviation for sample repeatability or method precision also lie well within the limits (% RSD ≤ 2.0) indicating the sample repeatability of the method [Table 2].

Table 1: System suitability studies results

S.No.	Experiment	Retention time	%RSD	Tailing Factor	Plate count
1	Method Precision/ Specificity/SIAS	3.099	0.34	1.00	3307
2	Linearity/Accuracy	3.155	0.05	1.00	3226
3	Ruggedness	3.038	0.21	0.99	4153
4	Stress Study	3.043	0.62	1.08	3023

Table 2: Compiled data of method precision and ruggedness

S.No.	% Assay of Ivabradine	
	Method Precision	**Ruggedness**
1	98.3	98.3
2	97.1	97.8
3	97.6	98.7
4	98.1	97.9
5	97.4	97.6
6	98.1	98.8
Mean	97.8	98.2
SD	0.47	0.50
%RSD	0.48	0.51
Overall mean	98.0	
Overall SD	0.51	
Overall % RSD	0.52	

Specificity

There was no interference from placebo was observed at the retention time of Ivabradine hydrochloride peak. The peak purity plots indicate that peak of Ivabradine hydrochloride are pure and do not have any co eluting peaks. It shows the specificity of the method.

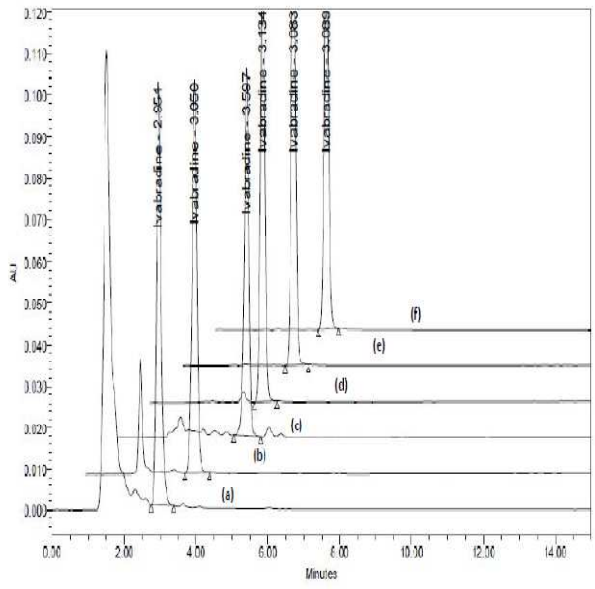

Fig. 3: Typical chromatogram of Ivabradine hydrochloride under stress condition. (a) Alkali hydrolysis (b) Oxidation (c) Thermal (d) Acid hydrolysis (e) Photolytic (f) Humidity

Force Degradation

The percentage degradation of Ivabradine hydrochloride in thermal and oxidation found to be 13 and 3 respectively.

The percent degradation for base hydrolysis, acid hydrolysis, photolytic and humidity was not found to be significant.

Using peak purity test, the purity of Ivabradine hydrochloride peak was checked for all degradation samples.

The peak purity plots show that the Ivabradine hydrochloride peak is homogeneous and that there are no co eluting peaks indicating that the method is stability indicating and specific [Fig. 3].

Linearity

The linearity of response for Ivabradine hydrochloride was determined over the range as shown in **Table 3**.The data indicates that the response is linear over the specified range [Fig. 4].

Table 3: Linearity of response for Ivabradine

S.No.	Conc.(µg/mL)	Peak area
1	70.698	684755
2	80.79	785378
3	90.89	878754
4	100.99	975855
5	111.09	1067855
6	121.19	1180209
7	131.29	1265446
Slope	9621	
Intercept	5262	
Correlation Coefficient	0.99974	

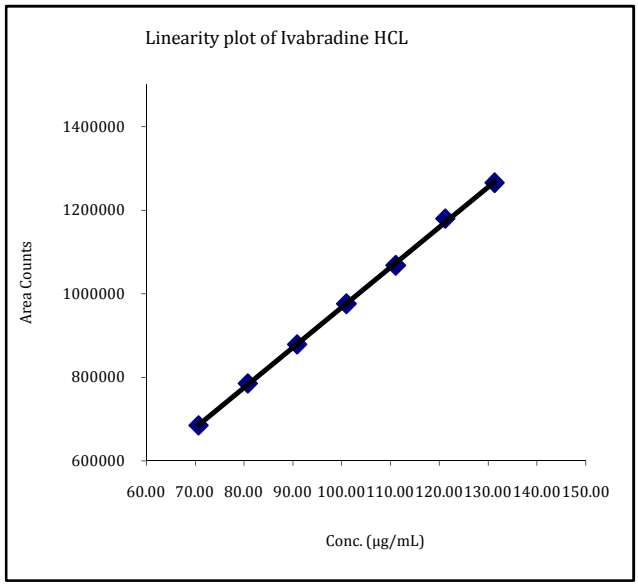

Fig. 4: Calibration curve of Ivabradine HCL

Accuracy

The accuracy proposed of analytical method was established by recovery studies. The data indicates that the method has an acceptable level of accuracy [Table 4].

Robustness

The compiled data for robustness is given in **Table 5**. Robustness of the method is indicated by the overall % RSD values between the control & data at each variable condition. The data shows the analytical method is not affected by deliberate variations such as flow rate, wavelength, mobile phase composition, pH and temperature and shows the robustness of method.

Ruggedness

The mean, standard deviation and %RSD for the two sets of data are shown in **Table 2**. Ruggedness of the method is indicated by the overall RSD values of 0.52 % for Ivabradine hydrochloride between the two sets of data. The data indicates the ruggedness of method is good.

Stability of the solution

Data for stability in analytical solution is shown with the cumulative % RSD up to 1478 mins. Meets the acceptance criterion, it is concluded that sample is stable in analytical solution for at least 24 h at room temperature.

Table 4: Accuracy data for Ivabradine

Recovery Level	Ivabradine		
	Amount added (mg)	Amount recovered (mg)	% Recovery
Level 1-80%, Rec 1	7.89	7.85	99.89
Level 1-80%, Rec 2	7.74	7.69	99.35
Level 1-80%, Rec 3	7.82	7.74	98.98
Level 2-100%, Rec 1	9.90	9.80	98.99
Level 2-100%, Rec 2	9.93	9.76	98.29
Level 2-100%, Rec 3	9.79	9.73	99.39
Level 3-120%, Rec 1	11.77	11.67	99.15
Level 3-120%, Rec 2	11.66	11.52	98.80
Level 3-120%, Rec 3	11.78	11.64	98.81
Mean			99.03
SD			0.373
RSD (%)			0.38

Table 5: Robustness data for Ivabradine

	Assay (%)									
S. No.	Control sample	Column oven Temp. [30°C]	pH Minus	pH Plus	Flow Minus	Flow Plus	Wave length Minus	Wave length Plus	Organic Minus	Organic Plus
1	98.3	98.5	98.3	99.0	98.0	98.0	98.2	98.2	98.5	99.0
2	97.1	97.6	97.9	98.7	97.3	96.8	97.0	97.0	97.2	97.3
3	97.6	97.7	98.8	99.5	97.7	97.4	97.5	97.7	97.7	98.1
4	98.1	-	-	-	-	-	-	-	-	-
5	97.4	-	-	-	-	-	-	-	-	-
6	98.1	-	-	-	-	-	-	-	-	-
Mean	97.8	97.9	98.3	99.1	97.7	97.4	97.6	97.6	97.8	98.1
SD	0.47	0.49	0.45	0.40	0.35	0.60	0.60	0.60	0.66	0.85
RSD (%)	0.48	0.50	0.46	0.40	0.36	0.62	0.62	0.62	0.67	0.87
Overall Mean		97.8	98.0	98.2	97.7	97.6	97.7	97.7	97.8	97.9
Overall SD		0.45	0.52	0.78	0.42	0.51	0.49	0.48	0.50	0.59
Overall RSD (%)		0.46	0.53	0.79	0.43	0.52	0.50	0.49	0.51	0.60

CONCLUSION

The developed analytical method is simple, rapid, accurate, precise, linear, rugged, robust and stability indicating. The method has an advantage, including rapid analysis, a simple mobile phase with mass compatibility and simple sample preparation. Hence this method can be used for routine quality control analysis and stability analysis of solid oral formulations.

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