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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF IVABRADINE HCL IN BULK AND FORMULATION

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Abstract: **A new simple, accurate, precise, sensitive and selective reverse phase high performance liquid chromatographic (HPLC) method has been developed and validated for the determination of Ivabradine HCl in bulk and tablet formulation. Chromatographic separation was performed on C18 (250 x 4.6 mm id, 5 µm) column using, ammonium acetate buffer pH (6.2) : Methanol (40:60 v/v) as mobile phase with flow rate 1ml/min at 281 nm with injection volume 10µl/ml. Retention time of Ivabradine HCl was found to be 6.9 min. The calibration curve was found to be linear between 40 to 80 µg/ml with regression coefficient 0.9974. The method is validated for specificity, linearity, accuracy, precision and robustness, found to be within the specified limit. The IV undergoes degradation under acidic, basic, oxidative, thermal and photolytic conditions. The LOD and LOQ for IH were 1.3µg/ml and 3.95µg/ml, respectively. The method was found to be robust with better accuracy and precision having % RSD value less than 2. The proposed method can be applicable for the estimation of Ivabradine HCL during routine analysis and stability studies.**

Keywords: **Ivabradine HCl, RP-High Performance Liquid Chromatography (HPLC), Validation, Forced degradation, ICH.**

I. INTRODUCTION

Pharmaceutical analysis plays a very important role in the examination of bulk drugs and formulations regarding the quality control and quality assurance. Analytical method development and validation is an important part of the development of new drug and formulation in pharmaceutical industry.[1] Ivabradine HCl is available under brand name corlanor having cardiotonic activity. The chemical name of ivabradine HCl is 3-(3-{[((7s)-3,4-Dimethoxybicyclo [4.2.0]octa-1,3,5-trien-7-yl)methyl]methylamino}propyk)-1,3,4,5-tetrahydro-2H-3-benzazepin-one, hydrochloride. The active substance is white to hygroscopic powder, soluble in water and methanol, practically insoluble in THF. Ivabradine HCl is a novel medication used for symptomatic management of stable angina pectoris. The drug act by reducing the heart rate.

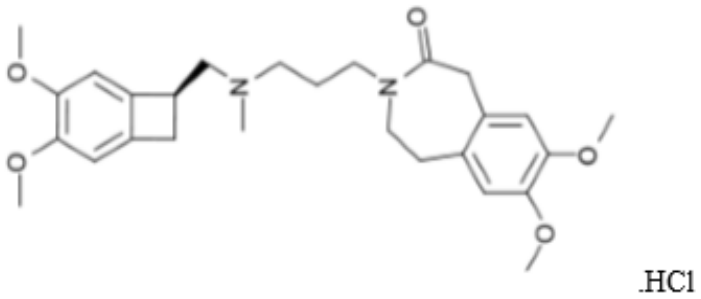

Fig.1: Structure of Ivabradine HCl

Literature survey reveals some spectrophotometric methods as well as chromatographic methods. The present article describes simple, accurate, precise, sensitive and selective analytical method for the estimation of ivabradine HCl in bulk and formulation.

II. MATERIALS AND METHODS

Material

Ivabradine hydrochloride was provided by Lupin Laboratories Ltd. Pune. Water and methanol were of HPLC grade and purchased from Research Lab Fine Chem Industries. Tablets used for study were purchased from local market.

A. Instrumentation

A JASCO EXTREMA LC SYSTEM-4000 with HIQSIL C18 (250 X 4.6 mm, id. 5µm) column.

B. Chromatographic conditions

The mobile phase, a mixture of ammonium acetate buffer (pH-6.2) and methanol (40:60 v/v) pumped at a flow rate of 1 ml/min through the column (C18; 250 x 4.6 mm id, 5µm) at 30^0c temperature. The mobile phase was degassed prior to use under vaccum by filtration through a 0.45 µm nylon membrane filter and sonicated for 10 min. Drug was analysed at 281 nm by PDA detector.

Column: C18; 250 x 4.6 mm id, 5µm

Wavelength: 281 nm

Column temperature: 30^0c

Flow rate: 1ml/min

Injection volume: 10µL

Run time: 15 min

Retention time: 6.9 min

C. Preparation of mobile phase

Ammonium acetate buffer of pH-6.2 and methanol of ratio 40:60 v/v were used as mobile phase. Both solutions were filtered through a 0.45 µm nylon filter and degassed by sonicating for10 min. Buffer was prepared by dissolving 0.308g Ammonium acetate in 200 ml of water for HPLC and sonicated for 10 min, volume was made up with HPLC grade water to 400ml. The pH of solution was adjusted to 6.2.

D. Preparation of diluents

Ammonium acetate Buffer of pH 6.2 and methanol (50:50) was used as diluent.

E. Preparation of standard solution

Accurately weighed 100 mg of Ivabradine HCl standard and transferred in a 100 ml volumetric flask and 50 ml of diluent was added to dissolve it. Sonicated for 10 min and volume was made up to the mark with diluent to produce 100 ml. 1ml of this solution was diluted to 10 ml with diluent and filter through 0.45 µm nylon injection filter before injecting in column.

F. Preparation of sample solution

10 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to a fine powder with the help of mortar and pestle. Accurately weighed powder equivalent to 100 mg of Ivabradine HCl and transferred to 100 ml volumetric flask, dissolved in 50 ml of diluent and sonicated for 10 min. Final volume was made up to 100 ml. The solution was filtered through watman filter. 1 ml of this solution was diluted to 10 ml with diluent to make solution of 100 ppm. Filter through 0.45µm nylon injection filter before injecting in column.

III. METHOD VALIDATION

A. Specificity

The specificity study included identification of drug peak and interference study. Injection of standard solution, sample solution and blank were analysed by direct comparison method to see that diluents or excipients were not interfering with the Ivabradine HCl peak.

B. Linearity

Accurately weighed 10 mg of Ivabradine HCl and transferred in 10 ml volumetric flask, diluted with diluent. From this solution linearity response of drug was determined over the range of 40 to 80 ppm for Ivabradine HCl. Linear relationship should be evaluated across the range of the analytical procedure. Linearity study performed for six times. Diluent was used as blank and calibration curves were made by plotting area Vs concentration.

C. Accuracy

The accuracy of the method was determined by spiked with known amount of drug in order to result in sample solution with the following concentration of drug in tablet, representing 80%, 100% and 120% of drug relative to the working concentration in triplicate according to the method of analysis and analyzed as per the method. % recovery was calculated.

D. Precision

Method precision

Method precision of the analytical method was determined by analyzing six samples of same concentration prepared separately. The % RSD of drug response was calculated for the six injection samples.

System precision

System precision of the analytical method was determined by analyzing standard solution. The % RSD of drug response was calculated for the six replicate injections.

E. LOD & LOQ

Six sets of linearity concentrations were analyzed and LOD & LOQ were calculated.

LOD= 3.3 x S.D/ slope

LOQ= 10 x S.D/slope

F. Robustness

The robustness of an analytical method is its capacity to remain unaffected by small changes in method parameters. The parameters like wavelength, flow rate, mobile phase pH, mobile phase concentration and column temperature were changed one by one in triplicate and their effect was observed on system suitability.

G. Degradation study

***Acid degradation*:-** To 1 ml of 1000 ppm solution of Ivabradine HCl, 1 ml of 0.1 N HCl was added in 10 ml of volumetric flask. Volume was made up with diluent to 10 ml and kept overnight. 1ml of this solution was diluted to 10 ml with diluent. Filtered through 0.45µm nylon injection filter and injected in HPLC column. Chromatogram of the solution was obtained and calculated for % of Ivabradine HCl.

***Alkaline degradation*:-** To 1 ml of 1000 ppm solution of Ivabradine HCl, 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask. Volume was made up with diluent to 10 ml and kept overnight. 1 ml of this solution was diluted to 10 ml with diluent. Filtered through 0.45 µm nylon injection filter and injected in HPLC column. Chromatogram of the solution was obtained and calculated for the % of Ivabradine HCl.

***Oxidative degradation*:-** To 1 ml of 1000 ppm solution of Ivabradine HCl, 1 ml of 30% of H_2O_2 was added in 10 ml of volumetric flask. Volume was made up with diluent to 10 ml anf kept overnight. 1 ml of this solution was diluted to 10 ml with diluents. Filtered through 0.45 µm nylon injection filter and injected in HPLC column. Chromatogram of the solution was obtained and calculated for the % of Ivabradine HCl.

***Thermal degradation*:-** Sample of Ivabradine HCl was kept in the petridish and expose to the temperature 70°c for 3 hrs in oven. After 3 hrs 10 mg of Ivabradine HCl was weighed and transferred to 100 ml volumetric flask. Dissolved and diluted with diluent. Solution of 10 µg/ml was prepared from this solution. Filtered through 0.45 µm nylon injection filter and injected in HPLC column. Chromatogram of the solution was obtained and calculated for the % of Ivabradine HCl.

***Photolytic degradation*:-** Sample of Ivabradine HCl was kept in the petridish and expose to the UV radiation for photostability in UV chamber for 2 hrs. After 2 hrs 10 mg of Ivabradine HCl was weighed and transferred to 100 ml volumetric flask. Dissolved and diluted with diluent. Solution of 10 µg/ml was prepared from this solution. Filtered through 0.45 µm nylon injection filter and injected in HPLC column. Chromatogram of the solution was obtained and calculated for the % of Ivabradine HCl.

IV. RESULTS AND DISCUSSION

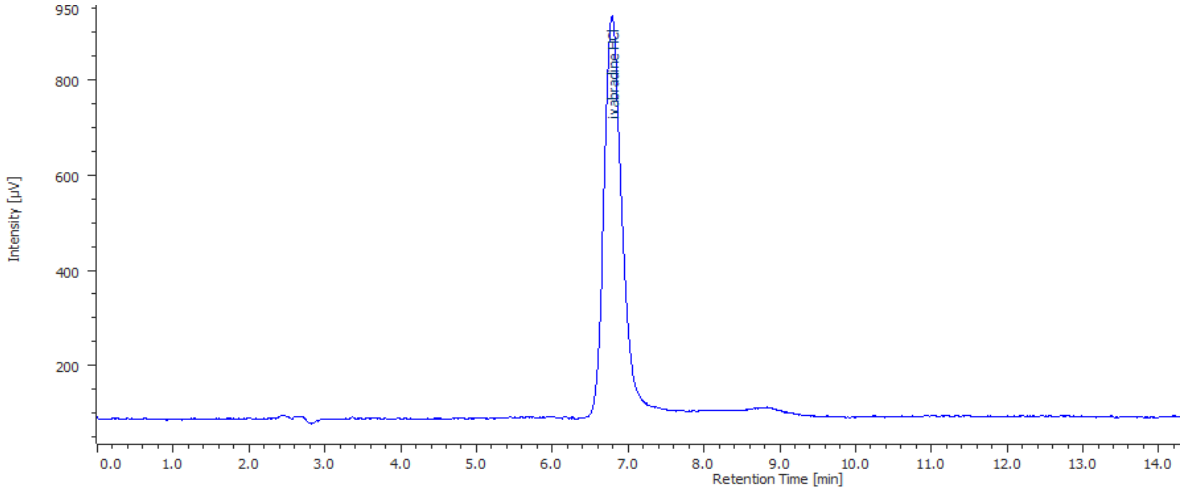

Fig. N: Chromatogram of Ivabradine HCL

Specificity

No peaks obtained at the retention time of Ivabradine HCl of blank and placebo.

Retention time of Ivabradine HCl is 6.9 min.

Linearity

TABLE I: LINEARITY DATA

Concentration (ppm)	**Area**
40	9480
50	12126
60	14878
70	17813
80	19875
Cc	**0.9974**
Y-intercept	**Y=264.77x-1051.8**

Accuracy

TABLE II: ACCURACY DATA

Sample	**Amount added (mg)**	**Amount found (mg)**	**% Recovery**	**Average**	**% RSD**
80%	48.12	47.61	98.92	99.19	0.3774
	48.12	47.66	99.04		
	48.12	47.94	99.62		
100%	60.16	59.75	99.31	99.29	0.37305

	60.16	59.50	98.91		
	60.16	59.95	99.65		
120%	72.19	71.94	99.65	99.24	0.35229
	72.19	71.51	99.06		
	72.19	71.49	99.03		

Precision

TABLE III

Sample-ID	**Area**	
	System precision	**Method precision**
1	13186	13186
2	12944	13510
3	12976	13026
4	12960	13169
5	13336	13441
6	13285	13231
Average	**13114.5**	**13260**
S.D	**176.2768**	**181.4461**
%RSD	**1.3441**	**1.36**

Robustness

TABLE IV: ROBUSTNESS DATA

Sr. No	**Normal**	**Low flow (0.8)**	**High flow (1.2)**	**λmax (279) nm**	**λmax (283) nm**	**pH (5.9)**	**pH (6.4)**	**MP 35:65**	**MP 45:55**	**Temp (25°c)**	**Temp (35°c)**
1	100.40	98.30	97.90	101.00	100.30	99.28	99.50	100.40	99.41	99.82	100.40
2	100.33	99.80	99.60	100.60	100.01	100.10	99.80	99.82	100.00	98.72	100.10
3	99.64	99.50	99.90	100.06	99.48	99.82	100.00	99.68	99.63	99.68	99.94
Mean	**100.12**	**99.20**	**99.10**	**100.55**	**99.93**	**99.73**	**99.70**	**99.96**	**99.68**	**99.07**	**100.14**
S.D	**0.42004**	**0.79**	**1.07**	**0.4717**	**0.4158**	**0.4168**	**0.25**	**0.381**	**0.298**	**0.527**	**0.2335**
% RSD	**0.4195**	**0.80**	**1.08**	**0.4691**	**0.4161**	**0.4179**	**0.25**	**0.38**	**0.229**	**0.532**	**0.2331**

Force degradation

TABLE V: FORCE DEGRADATION

Condition		**% Recovery**
Acid	0.1 N HCl	90.60
Base	0.1 N NaOH	76.66
Oxidative	3 % H_2O_2	96.56
Heat	70^{O}c	72.89
Photolytic	UV	97.24

V. SUMMARY OF VALIDATION STUDY

TABLE VI: SUMMARY OF VALIDATION STUDY

Validation parameters	**Results**
Linearity equation	Y=264.77x-1051.8
Correlation coeffitient	0.9974
LOD	1.3 µg/ml
LOQ	3.95 µg/ml

VI. CONCLUSION

The developed method was found to be simple, accurate, precise, sensitive and selective for analysis of Ivabradine HCl in bulk and formulation without any interference from the excipients. The method was developed as per ICH guidelines. The λmax of Ivabradine hydrochloride was found to be 281 nm. The mobile phase used for analysis was acetate buffer: methanol (40:60 v/v), using C_{18}(250 x 4.6mm id, 5µm) column as stationary phase, having flow rate of 1ml/min with injection volume 10µl/ml. This method is validated for specificity, linearity, accuracy, precision and robustness, found to be within specified limit. Stability indicating method was performed by influence of acid, alkaline, oxidative stress, thermal stress and photolytic stress. This method gives better separation with good retention time and can be used successfully for routine analysis. The developed method effectively separates IV from its degradation products. This method may be used for monitoring the stability of Ivabradine HCl.

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