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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF IVABRADINE HYDROCHLORIDE (ANTIANGINAL) DRUG

Suresh Kumar Yadav*, Dr. C. Sreedhar, T. Sreenivasa Rao, Akkamma H. G.

Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, #33/2, Thirumenahalli, Hegde Nagar Main Road, Bangalore– 560064, KA. India.

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***Corresponding Author**
Suresh Kumar Yadav
Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, #33/2, Thirumenahalli, Hegde Nagar Main Road, Bangalore– 560064, KA. India.

ABSTRACT

Chromatography was performed by isocratic reverse phase separation using a Agilent Eclipse Plus C_8 column of particle size 5µ, (150×4.6mm). The separations were achieved at the UV detection at 281nm using the mobile phase of Tris buffer: Acetonitrile in the ratio of 25:75. Flow rate was 1ml/min and the injection volume was set at 20 µl with 10mins of runtime. The retention time was observed at 5.13 mins for Ivabradine Hydrochloride. The method was validated by using various validation parameters like accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ). The standard curve was linear over a working range of 10-60 µg/ml and gave an average correlation factor 0.9936 for Ivabradine Hydrochloride. The limit of detection and the limit of quantification were found to be 1.07 µg/ml and 3.57 µg/ml for Ivabradine hydrochloride respectively. The method showed good recoveries and relative standard deviations of intra and inter day assay less than 2. This method can be easily and conveniently used for routine analysis of Ivabradine Hydrochloride in bulk and tablet dosage forms.

KEY WORDS: Ivabradine Hydrochloride, RP-HPLC, Accuracy, Precision, Linearity, LOD, LOQ.

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 thesymptomatic 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 betablocker 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]

Chromatographic methods have been described for the quantitative determination of Ivabradine Hydrochloride in formulations as well as biological fluids. These include Spectroscopy[6] and high performance liquid chromatography.[7-9] These previous published methods comprise of complicated mobile systems and are not directly applicable for this novel type of dosage form which is prepared and need more investigation for method development and validation. Therefore, the main aim of this work was to develop and validate a stability indicating RP HPLC method for estimation of Ivabradine Hydrochloride from a novel orally disintegrating tablet containing pellet formulation.

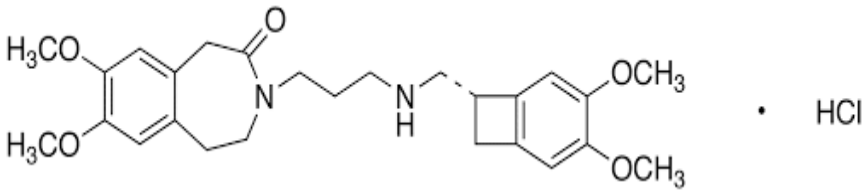

Figure 1- Chemical structure of Ivabradine Hydrochloride.

MATERIALS AND METHODS

Reagents and chemicals

Ivabradin is a tablet dosage form each contains 5mg of ivabradine hydrochloride. HPLC grade Acetonitrile (Merck), Analytical grade Tris buffer was used as the solvents throughout the experiment. Pharmaceutical formulation Ivabrad tablet (label claim contain 5mg) was used in HPLC analysis. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

Instrumentation

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on Agilent Eclipse plus C_8

column (5µm 4.6x150mm), column length is 25 cm with UV detection at 281nm. Analytical weighing balance. (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON230VAC, 50Hz), vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam uv-visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-dual channel was used for acquisition, evaluation and storage of chromatographic data.

Chromatographic condition

After several trials with the different combination and ratio of solvents, the mobile phase tris buffer (buffer): Acetonitrile (25:75v/v) at P^H-8.3. Retention time (R_t) 5.13 min for Ivabradine Hydrochloride. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component shows reasonably good response and maximum peak at 281nm.

Standard solutions for HPLC estimation of Ivabradine hydrochloride

20 tablets are taken, powdered and average weight is taken. 5mg of active ingredient is transferred into 10 ml of volumetric flask and is dissolved in mixture of acetonitrile and Tris buffer(75:25) volume were made up to the mark with same solvent This gave the concentration of 200 µg ml^{-1} of Ivabradine hydrochloride (Stock-1) . From stock solution 1, 6 dilution was prepared between 10-60 µg ml^{-1} which is working concentration.

RESULTS AND DISCUSSIONS

Method development

The developed method was validated according to ICH guidelines [4] with respect to specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) ruggedness, robustness and system suitability.

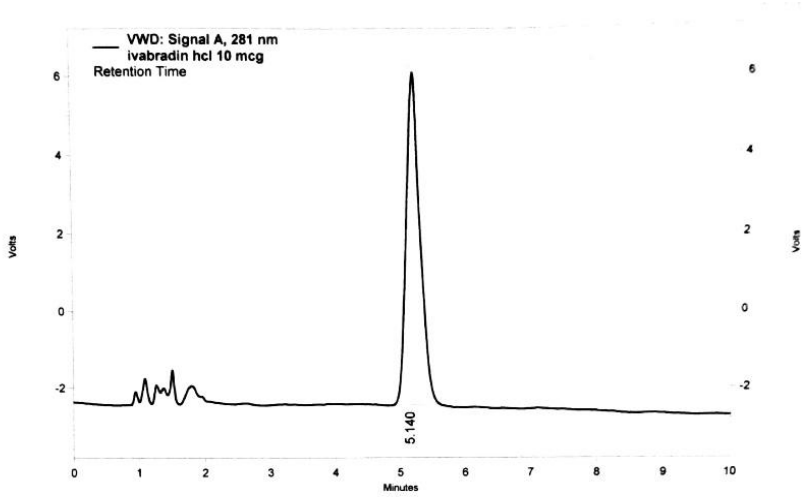

Figure 2- A typical chromatogram for Ivabradine Hydrochloride.

Linearity and range

By using the working standard, aliquots of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, were prepared with acetonitrile and buffer mixture. Six dilutions of each of the above mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration were injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

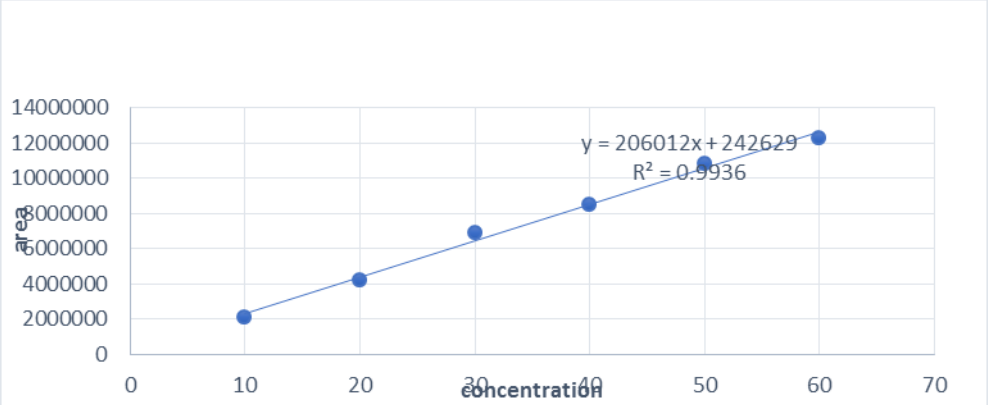

Figure 3- linearity graph of Ivabradine Hydrochloride.

Table 1: Linearity data for Ivabradine hydrochloride.

Concentration (μgml^{-1})	Area
10	2093398
20	4199908
30	6893970
40	8507627
50	10779718
60	12243594

Precision

The precision of the assay was determined in terms of intra and inter day variation in the peak area for a set of drug solution 30µg/ml, assayed six times on the same day and on different 2 days.

The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean with
100(CV=SD/MEAN × 100) shown in the graph.

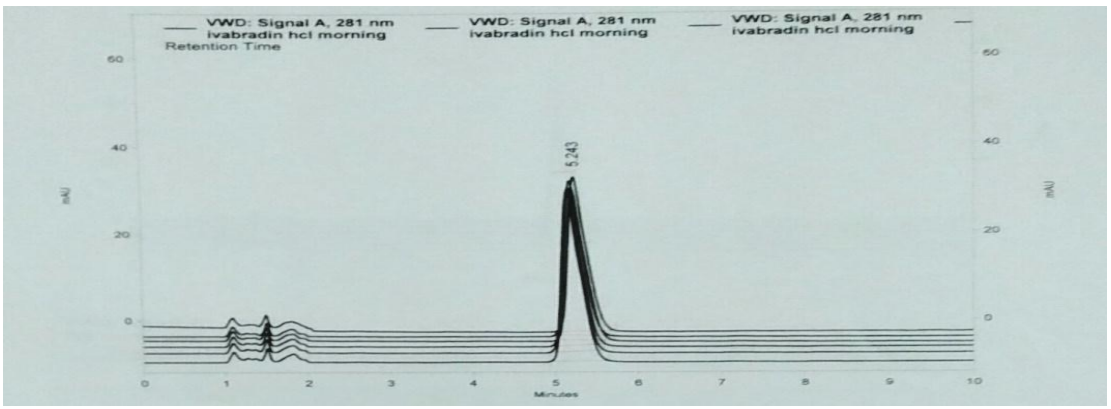

Figure 4 - Chromatogram showing intraday precision –morning.

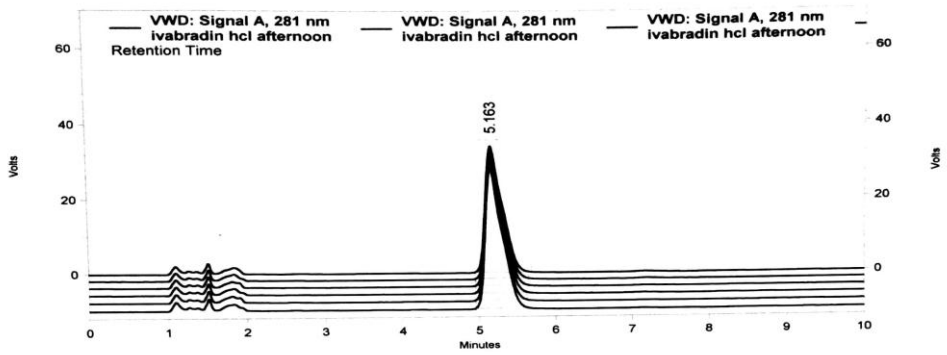

Figure 5 - Chromatogram showing intraday precision –afternoon.

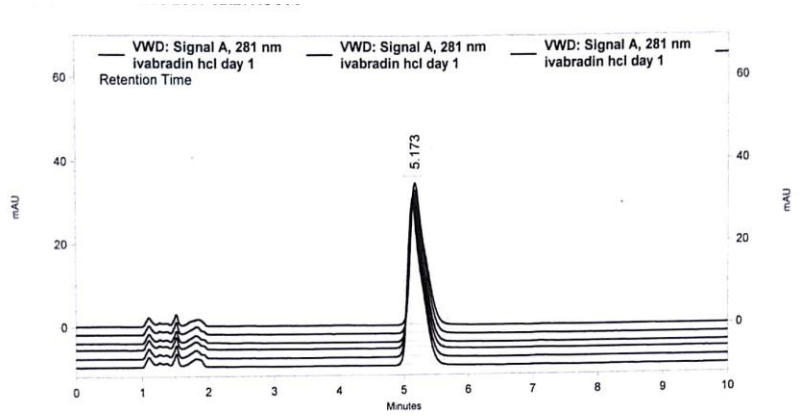

Figure 6: - Chromatogram showing inter day precision (Day-1).

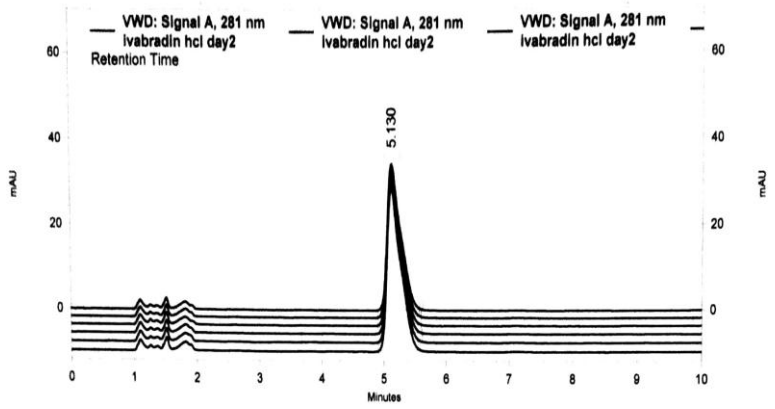

Figure 7: - Chromatogram showing inter day precision (Day-2).

Accuracy

The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the acetonitrile.

For 80% Accuracy for ivabradine hydrochloride:

5mg of the pure drug was added to 4mg of formulation

For 100% Accuracy for ivabradine hydrochloride:

5mg of the pure drug is added to 5 mg of formulation

For 120% Accuracy for ivabradine hydrochloride:

5mg of the pure drug is added to 6 mg of formulation

Table 2: - Accuracy data for estimation of Ivabradine hydrochloride.

Sl.no	Level of percentage recovery	Amount present (mg/tablet)	Amount of standard drug added	Area response	Mean	SD	RSD(%)	Total amount recover	% recovery
1	80%	5	4	8507627 8587526 8605027	8566726.7	51924.457	0.6	40.27	100.67
2	100%	5	5	10779718 10987812 10888721	10885417	104086.34	0.95	50.49	100.98
3	120%	5	6	12243594 12434495 12349594	12342561	95644.63	0.77	60.48	100.8

Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, wavelength ±5nm results are shown.

Table 2: Robustness of Ivabradine Hydrochloride.

Sl.no.	Parameter	Optimized	Used	Retention time(mins)
1	Flow rate	1 ml/min	0.9 ml/min	5.823
			1.1ml/min	4.767
2	Detection wavelength	281 nm	279nm	5.223
			283 nm	5.223

CONCLUSION

From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient and cost effective for the estimation of Ivabradine Hydrochloride in oral solid dosage form. The proposed method has a run time of 10mintues, which makes the method simple, cost effective and suitable for the routine analysis of Ivabradine Hydrochloride in oral solid tablet dosage form.

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REFERENCES

1. Parfitt K. Martindale. The complete drug reference. London:Pharmaceutical Press; 2007. p.1185.
2. O'Neil MJ. The Merck Index: an Encyclopaedia of Chemicals,Drugs and Biologicals. 14th ed. Whitehouse Station: MerckResearch Laboratories, Division of Merck and Co; 2006. p.907.
3. Ivabradine hydrochloride: Scientific discussion,http://www.emea.europa.eu/humandocs/ PDFs/EPAR/corlentor/32123005en6.pdf [accessed on 10/03/2013]
4. Summary of Product Characteristics, http://drugsabout.com/drugs/procoralan/procoralan.pdf. [Accessed on10/03/2013]
5. Summary of Product Characteristics.http://www.emea.europa.eu/ humandocs/ PDFs / EPAR/procoralan /H-597-PI-en.pdf.[accessed on 10/03/2013]
6. Shweta Maheshwari, Amit P. Khandhar, Anurekha Jain. Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form. Eurasian J. Anal. Chem. 2010; 5(1): 53-62.
7. Md. Rezowanur Rahman, Md. Asaduzzaman, S. M. Ashraful Islam. Development and validation of RP-HPLC method for analysis of Ivabradine Hydrochloride in tablet dosage forms. RJPBCS. July-Sept 2012; 3(3): 1032-1043.
8. Selva kumar P, Pandiyan K, Rajagopal K. Development and validation of stability indicating rapid HPLC method for estimation of Ivabradine hydrochloride in solid dosage form. Int J Pharm Pharm Sci. 2014; 6(4): 378-382.
9. Sunitha Seerapu, B. P. Srinivasan. Development and Validation of RP-HPLC Method for the Estimation of Ivabradine Hydrochloride in Tablets.Indian Journal of Pharmaceutical Sciences. Sept-Oct 2010; 72(5): 667-671.