IJMPR 2025, 9(6), 61-68

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 6.669

DEVELOPMENT AND VALIDATION OF NOVEL ANALYTICAL METHOD FOR IVABRADINE IN BULK AND DOSAGE FORM BY UV VISIBLE SPECTROPHOTOMETRY

Bindu R.*, Alakananda M. R., Adhila Samad, Geethika Ashok, Krishnapriya E., Vaishnavi S., Ayda Cherian, Shyni Bernard

College of Pharmaceutical Sciences, Govt. Medical College, Kottayam Kerala University of Health Sciences, Thrissur.

Article Received on: 02/04/2025
Article Revised on: 23/04/2025
Article Accepted on: 13/05/2025

***Corresponding Author**
Bindu R.
College of Pharmaceutical Sciences, Govt. Medical College, Kottayam Kerala University of Health Sciences, Thrissur.

ABSTRACT

This research centers on the establishment and validation of a novel analytical approach for the quantifying Ivabradine in bulk and dosage forms employing UV-visible spectroscopy with Ferric phenanthroline as a complexing reagent. Ivabradine, an antianginal drug, is crucial for managing chronic stable angina and heart failure by blocking the HCN channel in the sinoatrial node (SAN) of the heart. The study outlines the method's optimization, including the determination of optimal reagent volumes and heating times, to enhance the sensitivity and accuracy of the analysis. A calibration curve was established, demonstrating a linear relationship between absorbance and concentration across the 2–8 µg/ml range, with a correlation coefficient of 0.9996. The molar absorptivity was calculated at 0.10626, indicating a reliable method for quantifying Ivabradine. Validation parameters such as accuracy, precision, and robustness were assessed, yielding recovery rates between 97-102% and relative standard deviations below 2%. The method was environmentally friendly, aligning with green analytical chemistry principles. This study provides a cost-effective and efficient approach to routine quality control of Ivabradine in pharmaceutical formulations.

KEYWORDS: Ivabradine, Ferric phenanthroline, UV- Visible spectrophotometry, validation, Greenness.

INTRODUCTION

Ivabradine, formally known as 3-[3-({[(7S)-3,4-dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl]methyl}(methyl)amino)propyl]-7,8-dimethoxy-2,3,4,5-tetrahydro-1H-3 benzazepin-2-one, is a derivative belonging to the Benzazepinone class. Physically, it appears as a white to slightly yellow powder that shows solubility in both water and 0.9% saline solution.

As a pharmacological agent, Ivabradine reacts by lowering the heart rate. It attains this by selectively curbing the cardiac pacemaker current (If). This current, distinguished as a mixed sodium-potassium inward flow, plays a vital function in modulating the spontaneous diastolic depolarization within the sinoatrial (SA) node, thereby altering the heart's overall rate. The abstract additionally notes Ivabradine's significance as an antianginal drug used in managing chronic stable angina and heart failure, specifically mentioning its action of blocking the HCN channel in the sinoatrial node (SAN).

The literature review reveals that almost all the methods developed for Ivabradine hydrochloride involve RP-HPLC, LC-MS/MS etc. However, no UV spectroscopic determination method using Ferric phenanthroline reagent has been reported in recent times. Consequently, the primary goal of the present investigation is to establish a novel analytical method for Ivabradine in bulk material and target dosage forms. This study aims to develop a method that is simple, accurate, reproducible, and economical. The validation of this newly developed method has been conducted following the guidelines set forth by ICH and FDA.

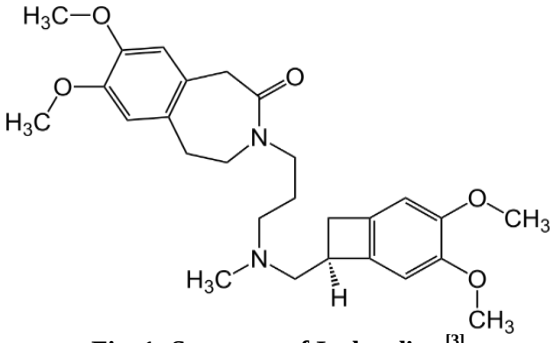

Fig. 1: Structure of Ivabradine.[3]

MATERIALS AND METHODS

Ivabradine hydrochloride bulk powder obtained from NIFTY LABS Pvt LTD. off:203, Satya residency, Plot No .7-1-54/1, Ameerpet, Hyderabad-16, Batch number: ID0030123. Commercially available pharmaceutical

dosage form Ivabradine Tablets (5mg)(Jan Aushadhi), Pharmaceutical and Medical Devices Bureau of India, Videocon Tower, Jhandewalan Extension, New Delhi-110055, Batch Number: MT241737. 1, 10-Phenanthroline Hydrate obtained from Spectrum Reagents and Chemicals Pvt. Ltd, Cochin, Kerala, India. Ammonium Ferric Sulphate GR obtained from Citra Laboratory Reagents, Cochin 25. Hydrochloric Acid GR obtained from universal chemicals and scientific industries, Haripad, South India-690514. Distilled water.

INSTRUMENTS

Shimadzu analytical balance, Amkette Analytic Ltd was used to take weights. Absorption spectral measurements were carried out using Cary Series UV- Vis Spectrophotometer[4], Agilent technologies. A Sonicator and a Centrifuge also were used.

PREPARATION OF STANDARD SOLUTION

Weighed 100mg Ivabradine reference sample and transferred it into a 100ml volumetric flask. Dissolved the sample up to the mark with distilled water(Solution A: 1000 µg/ml). From solution A transferred 1ml to another 10 ml volumetric flask and made up the volume with Distilled Water (Solution B: 100 µg/mL).

COMPLEX CHEMISTRY

Fe3+ form obtained from the reaction of 1,10-phenanthroline and ferric ammonium sulfatedodecahydrate. Primarily Fe3+ion causes the oxidation of Ivabradine and Fe3+ ion itself is reduced into Fe2+ ion. The latter ion complexes with 1,10-phenanthroline and produces a red color. Ivabradine is estimated by measuring the intensity of red colored complex (ferroin) using UV-visible spectroscopy.[5]

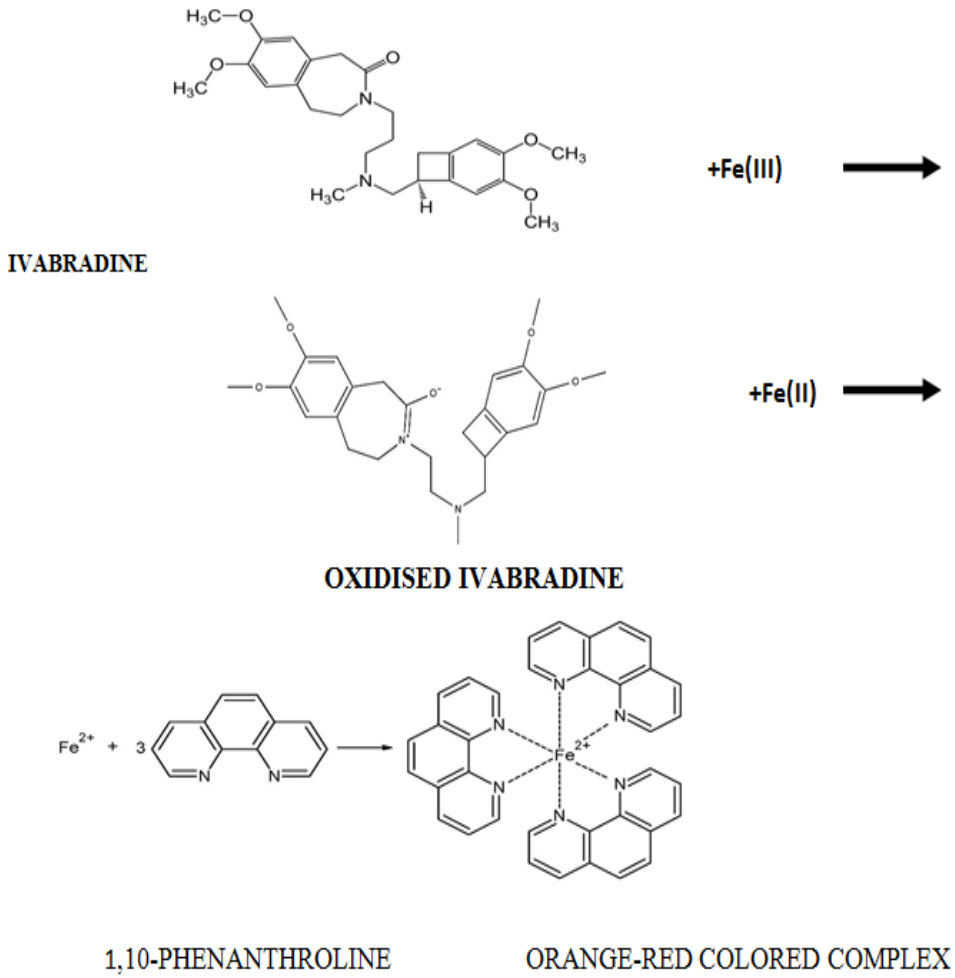

Fig. 2: Reaction of Ivabradine with Ferroin.

SELECTION OF λ_{max}

Prepared 8µg/ml solution by taking 0.8ml of 100 µg/ml of Ivabradine solution into a test tube. Added 3ml of Ferric phenanthroline reagent successively. The prepared solutions underwent heating in a boiling water bath for a duration of 20 minutes. Following heating, the solutions were cooled, transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark with distilled water. Performed initial correction of UV- instrument using blank. Solutions containing 3 ml of Ferric phenanthroline reagent heated for 20 minutes are taken as blank. Scanned 8µg/ml in visible regions ranging from 400 nm to 800 nm. Absorption spectra showed λ_{max} at 510 nm.

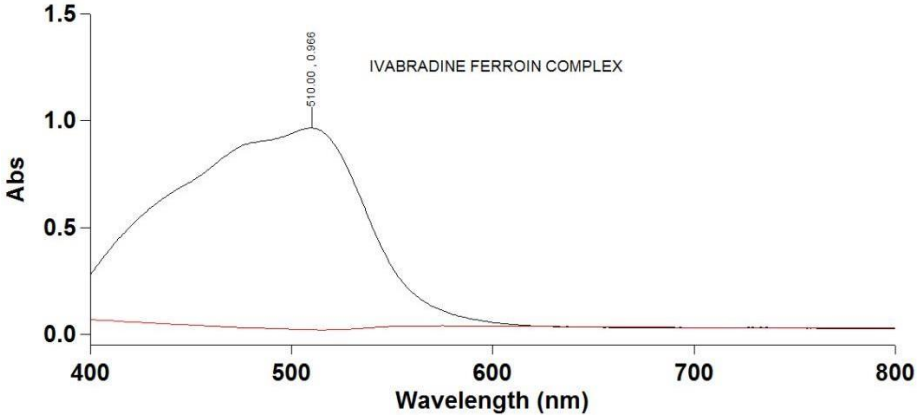

Fig. 3: The absorption spectrumof the Ivabradine-ferroin complex.

OPTIMIZATION OF HEATINGTIME AND VOLUME OF REAGENTS FOR NEW METHOD DEVELOPED

a) Optimization of the volume of Ferric – phenanthroline reagent

Different volumes of ferric phenanthroline reagent are taken for the study. Accurately pipetted out 0.8 ml from 100µg/ml solution of Ivabradine into four test tubes. Different volumes like 1, 2, and 3 ml of ferric phenanthroline reagent solution are pipetted out separately to every test tube. The solutions obtained were subjected to heating in a boiling water bath for 20 minutes. After this heating step, the solutions were allowed to cool, then transferred into four 10 ml volumetric flasks, and distilled water was added to reach the desired volume. The effect of varying volumes of ferric phenanthroline reagent in the absorbance of 8µg/ml of drug solution at 510 nm is observed. The absorbance of a solution is measured in triplicates and the mean absorbance is given in the table. A graph is also plotted which shows the relation between the volume of ferric phenanthrolinereagent and absorbance shown in Figure 4.[7]

Table 1: Absorbance data of different volumes of Ferric –Phenanthroline reagent.

Sl No	ConcentrationofIvabradine (µg/ml)	Volumeof Ferric - Phenanthroline reagent	Absorbance
1	8	1	0.5569
2	8	2	0.7013
3	8	3	0.8501
4	8	4	0.8501

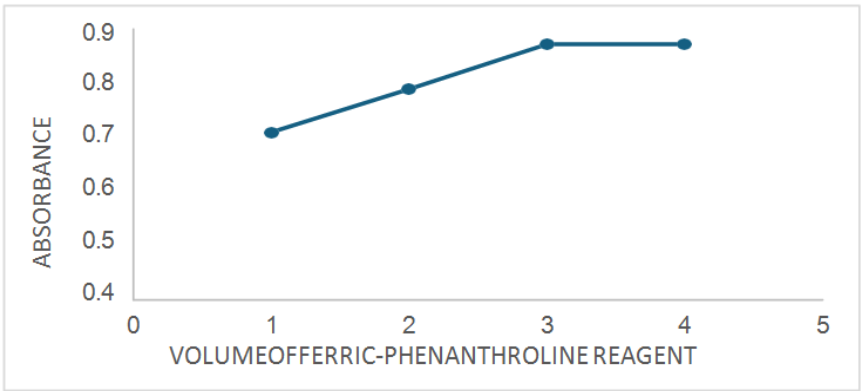

Fig. 4: Optimization of volume of Ferric-Phenanthroline reagent.

The absorbance of 8 µg/ml of Ivabradine drug solution increases initially with the increase in volume of ferric phenanthroline reagent. No further change in absorbance is observed after increasing the volume beyond 3 ml. Therefore, 3 ml of ferric phenanthroline reagent is optimized for the method.

b) Optimization of heating time: The experiment is conducted at various heating times including 0, 5, 10, 15, 20, 25, and 30 minutes. 0.8 ml of standard solution along with 3 ml of ferric phenanthroline reagent is taken in six test tubes. Each test tube is subjected to a different heating time. Once heated, the solutions in the test tubes were cooled. Each solution was subsequently transferred into one of six 10 ml volumetric flasks, and the final volume was adjusted to the mark using distilled water. The effect of changing heating time with absorbance at 510 nm

is recorded. The absorbance of a solution is measured in triplicates and the mean absorbance is given in the table. A graph showing the relation between heating type and absorbance is plotted in the figure.

Table 2: Absorbance data on different heating times of ferricphenanthroline reagent.

	HEATING TIME(min)						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Absorbance	0.3973	0.5234	0.6085	0.6776	0.8501	0.8501	0.8501

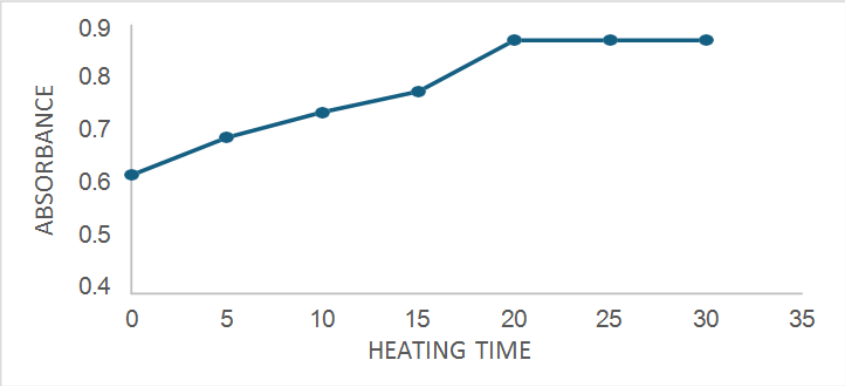

Fig. 5: Optimization of heating time.

Sample which is not heated shows less absorbance. Heated sample shows considerable absorbance. It is observed that increase in heating time of drug with the reagent initially increases the absorbance. Beyond 20 minutes, there is no further increase in absorbance is observed. Therefore, 20 minutes of heating is selected as the appropriate temperature for the developed method.

METHOD VALIDATION

Validation of the developed method was conducted as per ICH guidelines for the following key parameters[8]:

Linearity

The linearity of the developed method was assessed across a concentration range of 2 -8 µg/mL, with measurements taken at 510 nm. The resultant linear regression data is presented in Table 3.

Table 3: Linearity data.

Component	**Method parameters**			
Ivabradine	**Linearity**	**slope**	**intercept**	**R-value**
	2-8	0.0812	0.2006	0.9996

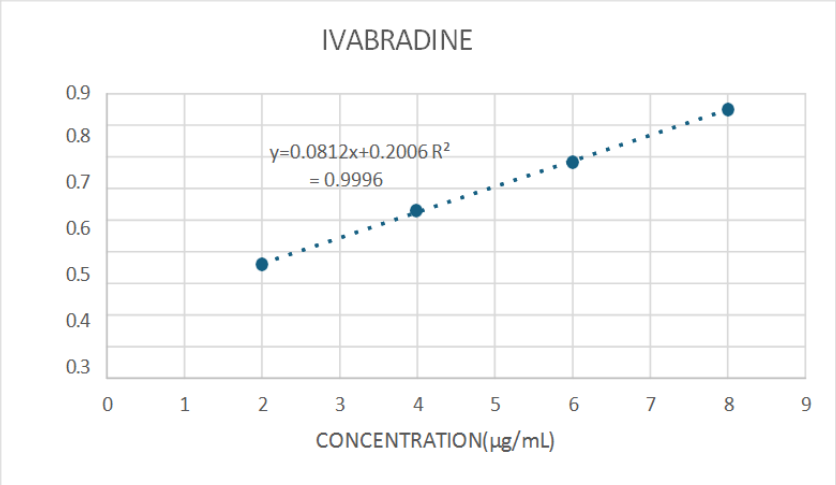

Fig. 6: Linearity data.

RECOVERY STUDIES

The accuracy of the developed method was assessed via recovery studies performed by the standard addition method. This entailed adding a known amount of standard to preanalyzed samples and subsequently reanalyzing the resultant solutions. Three determinations were conducted at each level. Absorbances were recorded at 510 nm, using water as the blank. The amount of drug recovered from the formulation was calculated, and the results are summarized in Table 4.

Table 4: Result of Recovery Study of Ivabradine.

Concentration of Drug solution (µg/ml)	Amount of tablet Powder present (mg)	Amount of standard added (mg)	Drug Recovery	
			mg	Percentage
2	12.5	12.5	12.25	98.02
	12.5	12.5	12.18	97.5
	12.5	12.5	12.23	97.9
4	12.5	12.5	12.7	101.6
	12.5	12.5	12.6	101.5
	12.5	12.5	12.6	101.5
6	12.5	12.5	12.37	99.03
	12.5	12.5	12.38	99.1
	12.5	12.5	12.4	99.2

Precision

Precision for an analytical procedure signifies the closeness of agreement (or degree of scatter) among results stemming from multiple analyses of a homogeneous sample performed under specified conditions. This method's precision was specifically assessed by determining its repeatability and Intermediate precisions.

Repeatability

Repeatability of the method was established through the analysis of six 8 µg/ml samples of Ivabradine test solution. Absorbances were measured at 510 nm, and the results are detailed in Table 5.

Table 5: Result of repeatability study-statistical validation data.

Component	The mean of % label claim	Standard Deviation (SD)	Relative Standard Deviation (%RSD)
Ivabradine	100.17	0.0768	0.0766

Intermediate Precision

The method's intermediate precision was assessed by analysing 8 µg/ml test solutions of Ivabradine at 510 nm. This evaluation involved conducting six replicate determinations across three consecutive days. Absorbance measurements of the solutions were recorded, and the results for each day are detailed in Table 6.

Table 6: Result of Intermediate Precision Study -Statistical Validation data.

Component	Mean of % labelclaim (n=18)	Standarddeviation (SD)	Relativestandard deviation (%RSD)
Ivabradine	100.28	0.08850612	0.08825882

Range

Range of the analytical procedure is acquired from linearity studies. Also Beer's law is obeyed in this range. From the linearity study, it is found that the range of the proposed analytical method is 2-8 µg/ml.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined using data derived from four sets of calibration curves that established the method's linearity. These values were calculated using specific equations.

$$LOD=3.3 \times \sigma/S$$

$$LOQ=10 \times \sigma/S$$

Where σ - standard deviation of the y-intercepts of the regression line

S- the slope of the calibration curve

Table 7: LOD AND LOQ data.

Drug	Wavelength (nm)	σ	S	LOD (µg/ml)	LOQ (µg/ml)
Ivabradine	510	0.006342	0.081155	0.257882	0.781461

STABILITY STUDIES

The absorbance of 8 µg/ml test solution conducted in triplicates at intervals of 0 min, 15 min, 30 min, 45 min, and 1 hour is measured at 510 nm in triplicates and the mean absorbance is given in the table below.

Table 8: Absorbance data on stability study.

Concentrationofdrug Solution(µg/ml)	8µg/ml				
Time(mins)	0	15	30	45	60
Absorbance at 510nm	0.8501	0.8501	0.8501	0.8501	0.8501

ESTIMATION OF IVABRADINE IN TABLET DOSAGE FORMS

Details of analyzed dosage forms:
Trade name: Ivabradine Tablets
Label claim: Ivabradine IP ……5mg
Mfg. by: Pharmaceuticals and Medical Devices Bureau of India (PMBI), New Delhi.

Weighed 20 tablets of Ivabradine. The average weight of one tablet of Ivabradine is determined. 20 tablets are then finely powdered using a clean mortar and pestle. A quantity of powder equivalent to 100 mg was accurately weighed. This powder was transferred to a 100 ml conical flask, and 100 ml of distilled water was added. The mixture was then sonicated for 30 minutes. Following sonication, the solution, referred to as Solution A, was filtered into a 100 ml volumetric flask using Whattman No. 1 filter paper. To prepare a 100 µg/ml solution, designated as Solution B, 1 ml of Solution A was transferred to a 10 ml volumetric flask, and the volume was made up to the graduated mark using distilled water. Subsequently, aliquots of 0.2 ml, 0.4 ml, 0.6 ml, and 0.8 ml were accurately pipetted from Solution B into four separate test tubes. To each test tube, 3 ml of Ferric phenanthroline reagent was successively added. The resultant solutions were then subjected to heating for 20 minutes in a boiling water bath. After heating and cooling, the solutions were transferred to volumetric flasks and their final volume was adjusted with distilled water. Measured the absorbance of four different concentrations of Ivabradine at 510 nm.[9]

Assay results are provided in Table 9

Table 9: Assay results of Ivabradine 5 mg.

Sl No	Concentration µg/ml	The amount presentper tablet Label claim (mg)	Amountobtained mg/tablet	Percentagelabel claim
1	2	5	4.8	97.5
2	4	5	5.1	102.3
3	6	5	4.9	99.3
4	8	5	4.9	99.9

COMPARATIVE STUDY USING FTIR

A comparative study using FTIR was conducted by comparing the spectra of pure Ivabradine standard and Ivabradine tablet samples, with the comparison based on figures. The FTIR spectrum of pure Ivabradine showed that all its characteristic peaks were present. It was observed that the FTIR spectra from the Ivabradine sample had similar absorption bands to the standard Ivabradine spectrum. The intensity (absorbance) of the peaks in the standard and sample spectra was observed and compared. Specifically, characteristic strong peaks were noted between 1700 and 1500 cm^{-1} in both the standard and sample spectra. These peaks are indicative of the carbonyl (-C=O) and amide group within the benzazepine-2-one ring. By comparing the intensity of these characteristic peaks in this specific region, a resemblance between the sample and the standard could be noted. However, it was also observed that the Ivabradine tablet sample displayed weaker peaks compared to the standard. This observation suggests that the sample's functional groups have weaker vibrational frequencies. Furthermore, this weaker intensity may potentially be due to degradation, resulting in a reduced amount of the active ingredient in the sample when compared to the standard. Therefore, the reduced intensity of the peaks was concluded to be a consequence of having a lesser amount of Ivabradine relative to the standard.[10]

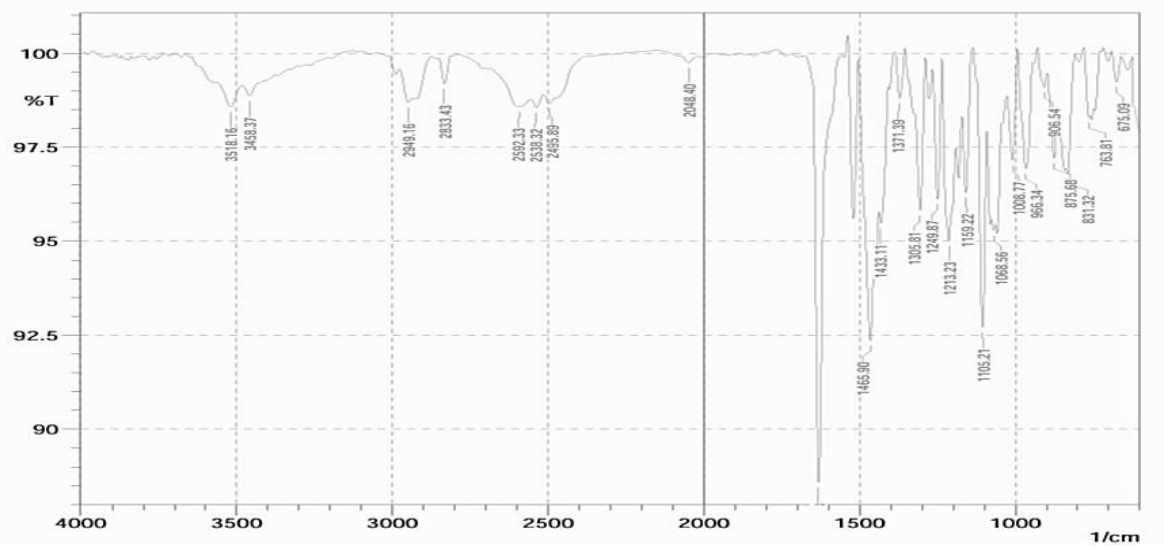

Fig. 7: FT IR spectrum of Ivabradine standard.

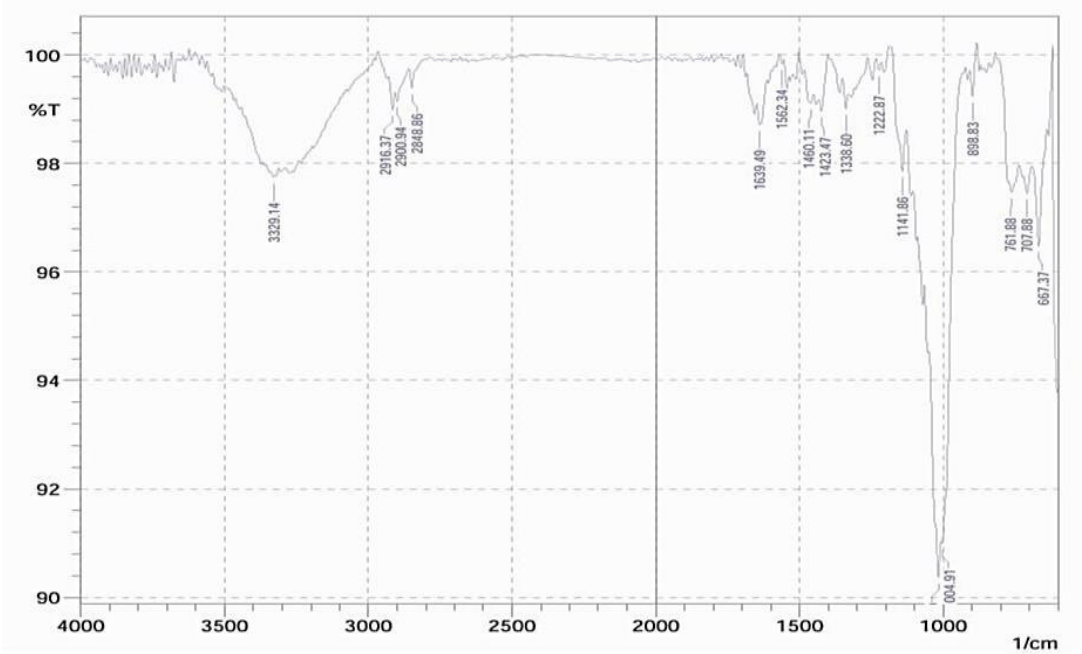

Fig. 8: FTIR spectrum of Ivabradine tablet 5mg (sample).

GREENNESS

The penalty points for the analytical method validation of Ivabradine which uses 3 ml of ferric phenanthroline solution, which generates less than 10 ml of waste containing phenanthroline calculated:

1. Penalty points for solvents
Toxicity of ferric phenanthroline
Low to moderate toxicity: 5 points
The amount used volume or mass of agent used < 10 ml: 1 point
2. Penalty points for waste generated 10 ml: 1 point
3. Penalty points for energy High-temperature operation: heated for 20 min: 5 points
4. Safety hazard: no risk
5. Complexation step: 5 points

Total penalty points = 17 points
Analytical eco scale score = 83 points

Interpretation: the method using Ferric phenanthroline is green but can be further improved by reducing the reagent amount or finding alternative, less toxic complexing agents.[11]

RESULTS AND DISCUSSION

By scanning 8µg/ml of Ivabradine in the variable range between 400 nm and 800 nm, the λmax was found to be 510 nm. Distilled water was used as the solubility solvent, and the linear concentration range for Ivabradine was 2 -8 µg/ml. The calibration graph correlation coefficient was determined to be 0.9996. The molar absorptivity was found to be 0.10626. The Ivabradine standard solution was found to be stable at different time intervals. Recovery studies were used to determine the accuracy of suggested approaches, which were shown to be 99.11% accurate. Repeated analysis of formulation and interday analysis were used to confirm the accuracy and the results showed that the % RSD was 0. 0766. The accuracy approach was verified and found to fall between 97 to 102 %. The correctness of this procedure was demonstrated by the standard deviation values, which were adequately low, and the RSD, which was less than 2 %. The obtained LOD and LOQ are 0.257882 and 0.781461 respectively. This suggested method was found to be green by the analytical eco scale method and GAPI method.

CONCLUSION

The UV spectrometric technique discussed in the current work has the following advantage: it is consistent, reliable, accurate, economical, efficient and applicable technique for analysingIvabradine tablets. This technique was developed for estimation of Ivabradine in bulk and pharmaceutical dosage forms(tablets). The validation procedure confirms that this is an appropriate method for their quantification in the formulation.

CONFLICTS OF INTEREST

The authors have no conflict of interest regarding this investigation.

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