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Development and Validation Of UV Spectroscopic Method For Estimation Of Ivabridine HCl In Tablet Dosage Form

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ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Ivabridine HCl in tablet dosage form. The drug is freely soluble in analytical grade water. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade water. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Ivabridine HCl in tablet dosage form and also validated as per ICH guidelines. The drug is soluble in analytical grade water, slightly soluble in methanol and freely soluble in ethanol. So, the analytical grade water is used as a diluent in method. The melting point of Ivabridine HCl was found to be 194-195˚C (uncorrected). It showed absorption maxima 260 nm in analytical grade water. On the basis of absorption spectrum the working concentration was set on 6µg/ml (PPM). The linearity was observed between 2-10 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.33 and 101.25% for three levels respectively. The % RSD for precision was found to be 0.54%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Ivabridine HCl in tablet dosage form. The method could be considered for the determination of Ivabridine HCl in quality control laboratories.

Keywords: Ivabridine HCl, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

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INTRODUCTION

Ivabradine hydrochloride is a new drug approved by the United State Food and Drug Administration (USFDA) in April 2015 for treatment of chronic stable angina pectoris in coronary artery disease [1, 2, 3]. Ivabradine is a specific heart rate lowering agent, acting by reducing the rate of pacemaker activity in the sinoatrial node. Chemically 3-[3- ({[(7S)-3,4-dimethoxybicyclo[4.2.0]octa-1, 3, 5- trien-7yl] methyl}methylamino)propyl]-1,3,4,5- tetrahydro-7,8-dimethoxy-2H-3-benzazepin -2- one and available in the form of hydrochloride salt [8, 9]. The active substance is white to hygroscopic powder, soluble in water and methanol, practically insoluble in THF [10, 11]. Ivabradine HCl is a novel medication used for symptomatic management of stable angina pectoris. Recent research suggests that it targets mainly on reducing the heart rate by decreasing myocardial oxygen demand [12]. Hence it is such that this drug had a wide scope of formulations to be developed for the effective treatment of chronic stable angina pectoris [13, 14]. This molecule selectively blocks the ʃ channel, which is responsible for initiation of the diastolic depolarization phase of the action potential, resulting in slower increase of the pacemaker current. The slower the increase of pacemaker current, the lower the heart rate [15]. Because of this mechanism, ivabradine has been effectively introduced into cardiac therapeutics.

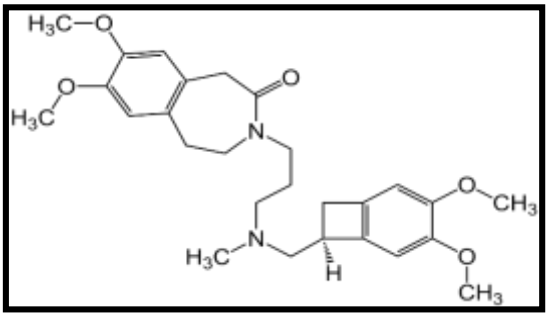

Figure 1: Chemical Structure of Ivabridine HCl

From literature review it's found that lot of work was done on HPLC [12-14] method development for Ivabridine Hydrochloride in combination with other drugs. But very few methods were reported on Ivabridine Hydrochloride tablets for UV method development.

The literature review reveals, few analytical methods reported for the determination of Ivabridine Hydrochloride individually, in various biological fluids [16-17] as well as dosage forms. Moreover, one method on derivative spectrophotometry for simultaneous estimation of Ivabridine Hydrochloride in their combined dosage form has also been reported [15]. This indicates that so far no UV method exists for the determination of these drugs in tablet dosage form in single.

MATERIALS AND METHOD

Instruments:

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

Materials:

Ivabridine HCl was obtained as a gift sample. Ivabridine HCl tablets were procured from local pharmacy. Water used was of analytical grade. Glass double distilled analytical grade water was used throughout the experiment. Freshly prepared solutions were employed.

Method development:

Determination of ƛ max (6 PPM)

20 mg weighed amount of Ivabridine HCl was dissolved into 100 ml of volumetric flask with analytical grade water. Pipette out 3 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with analytical grade water. This solution was subjected to scanning between 200-400 nm and absorption maximum was determine [4-8].

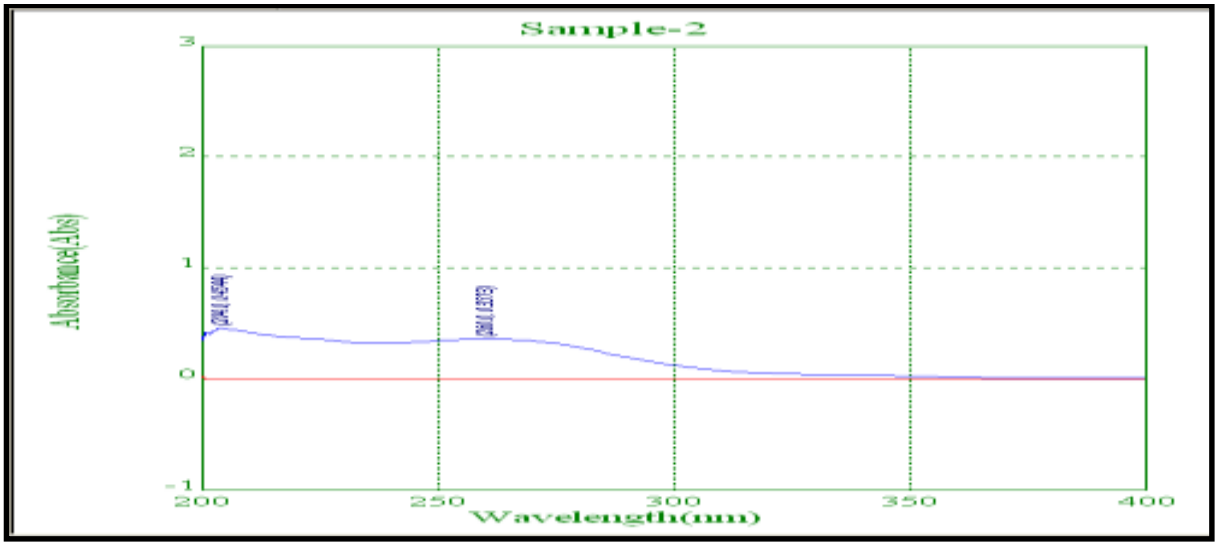

Figure 2: Calibration Curve

Preparation of Working concentration

Preparation of Standard stock solution:

Standard stock was prepared by dissolving 20 mg of Ivabridine HCl in 100 ml of analytical grade water to get concentration of 200 µg/ml (PPM).

Preparation of Standard solution:

Pipette out 3 ml from standard stock solution and diluted up to 100 ml with analytical grade water to get concentration of 6 µg/ml (PPM).

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Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade water. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution:

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution:

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Procedure for sample preparations [19,20]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 20 mg of Ivabridine HCl was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade water, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade water. Filtered the solution through whatmann filter paper. Pipette out 3 ml of the above solution and diluted up to 100 ml with analytical grade water. The absorbance was measured at 260 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form

Lupin Pharmaceutical Limited IVABRAD®(10 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.2695
3	Sample	0.2655

Table 2: Dosage Form Specifications

Type	Company	MFG. Date	EXP Date	Batch No.	Average weight (g)	Assay (%)
1	**IVABRAD®** **LUPIN Pharma (10 mg)**	12/06/2018	19/09/2021	CFD 145	0.1014	98.51

Method of validation [18]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity:

The linearity of the proposed assay was studied in the concentration range 2 - 10 PPM at 260nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies

Sr. no.	Sample Concentration	Absorbance
1	2 PPM	0.0971
2	4 PPM	0.1850
3	6 PPM	0.2812
4	8 PPM	0.3646
5	10 PPM	0.4355
Correlation coefficient		**0.997**

Accuracy:

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade water and measuring the absorbance at 260nm.

Table 4: Accuracy Studies

Spectrophotometric Method			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	4	3.95	98.75
100	6	5.90	98.33
120	8	8.10	101.25

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies

Sr. No.	Sample Solution	Absorbance
1	Sample solution-1	0.2684
2	Sample solution-2	0.2651
3	Sample solution-3	0.2682

4	Sample solution-4	0.2685
5	Sample solution-5	0.2683
Mean		0.2677
SD		0.0015
% RSD		0.54

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.2684	0.2668	**98.99**	**0.43**
		0.2651			
2	Analyst 2	0.2682	0.2684	**99.59**	
		0.2685			

Solubility of Ivabridine HCl

Solubility test was passed as per criteria.

Table 7: Results for solubility studies

Sr. No.	Title	Result
1	Analytical grade water	Soluble
2	Ethanol	Freely Soluble
3	Methanol	Slightly Soluble

Melting point of Ivabridine HCl

The melting point of Ivabridine HCl was found to be 194-195˚C (uncorrected).

Results for linearity for assay method of Ivabridine HCl

The linearity of method was determined at concentration level ranging from 2 to 10 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9972**

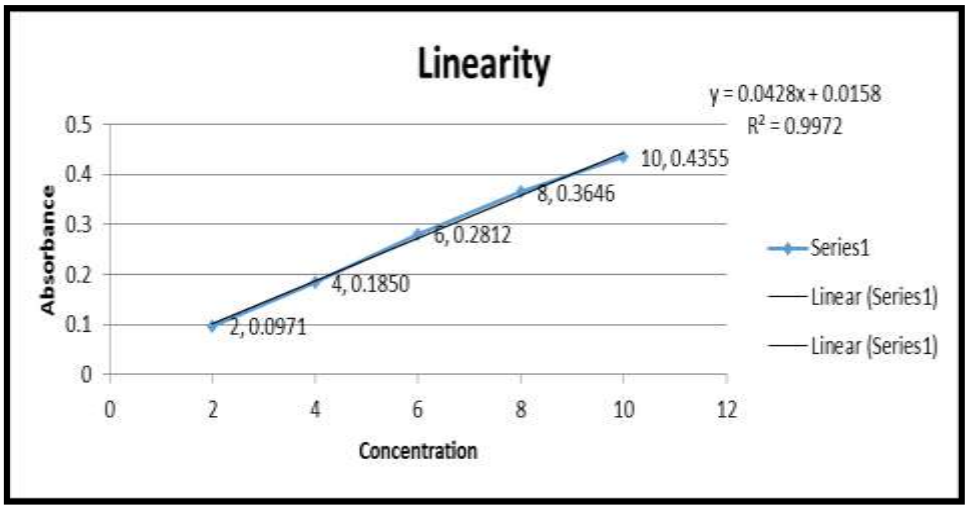

Figure 3: Ivabridine HCl Standard Curve

Results for accuracy for assay method of Ivabridine HCl

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

Results for precision for assay method of Ivabridine HCl

The % RSD for different sample of precision was found to be 0.54 and it is within acceptance criteria represented in Table - 5.

Results for ruggedness for assay method of Ivabridine HCl

The %RSD for different sample of ruggedness was found to be 0.43 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Ivabridine HCl in tablet form has been developed. From the spectrum of Ivabridine HCl, it was found that the maximum absorbance was 260 nm in analytical grade water. A good linear relationship was observed in the concentration range of 2-10 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Ivabridine HCl in solid dosage forms. Hence, the method could be considered for the determination of Ivabridine HCl in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. HBV - Hepatitis B virus
6. DNA - Deoxyribonucleic acid
7. HIV - Human Immunodeficiency Virus
8. ICH - International Council for Harmonization
9. RSD - Relative Standard Deviation
10. SD - Standard Deviation
11. Qty - Quantity
12. C - Celsius
13. M.D. - Manufacturing Date
14. E.D. - Expiry Date

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