IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Research Article

HPLC Method Development and Validation for the Quantification of Ivabradine in Tablets

Madhusudhana Reddy Induri* and Yarlagadda Urmila

Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Siddhartha Nagar, Vijayawada-520 010, Andhra Pradesh, India.

ARTICLE INFO

Article history:
Received 08 Feb 2021
Received in revised form 27 Feb 2021
Accepted 03 March 2021
doi.org/10.38111/ijapb.20210701003

Keywords:
Ivabradine; HPLC; ICH Guidelines; Quality Control

ABSTRACT

A precise and accurate HPLC method was developed for the quantification of Ivabradine in tablets. It was performed on Shimadzu LC-10AT VP system using C_{18} Column (150 x 4.6 mm; 5 µ). The mobile phase consisting of acetonitrile: 10 Mm ammonium acetate buffer (pH 7.2) in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min and eluents monitored at 285 nm. The method was validated as per ICH Q2(R1) guidelines. The retention time of ivabradine was 2.244 min. The calibration curve was constructed peak area versus concentration, which was linear from 10 - 50 µg/mL, had regression coefficient (r^2) greater than 0.999. The method had the mandatory precision, accuracy, and robustness for quantification of ivabradine in formulations. The developed method can be effectively employed in routine quality control for the quantification of ivabradine in tablets.

1. Introduction

Ivabradine (IBD), 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-one, is used in the treatment of chronic stable angina in patients unable to take β-adrenergic blockers, and in the treatment of heart failure. The mechanism of action of IBD is due to its interaction with receptor site of intracellular channel leading to disruption. This facilitates adequate oxygen supply and therefore by counters ischemia, due to imbalance between high input and decreased angina episodes[1-4]. A review of appropriate literature discovered that few HPLC[5-10], spectrophotometric[8,9,11,12] methods have been reported for the determination of IBD. However, there is no accurate and precise HPLC method reported for the estimation of IBD in pharmaceutical formulations.

2. Materials and Methods

Instrumentation: The Shimadzu LC-10AT VP system comprising of a binary gradient pump, an integral SIL-20AHT auto-sampler and UV-Vis SPD-10A VP detector. A Cyclo Mixer (Remi' Model: CM 101) was used for enhancing the dissolution of analyte in a diluent. An Ultra Sonicator (Loba Life; Model: 3.5L 100) was used for degassing of mobile phases. A digital pH meter (Systronics µ pH System 361) was used for pH adjustment. A Digital balance (Amkette Shimadzu; Model: ATX224) was used for weighing all the materials.

Materials: The working standard ivabradine oxalate is gifted by Glenmark Pharmaceuticals Limited, Mumbai. A marketable Ivabrad tablets (Lupin Pharma Ltd, Mumbai) containing IBD (10 mg) were bought from local pharmacies. The HPLC grade methanol, acetonitrile and water were obtained from Merck Life Science Private Limited, Mumbai. All other solvents and chemicals used were of analytical grade purchased from Loba Chemie, Mumbai.

* *Corresponding author.* Tel.: +91-7093819777.
E-mail address: imsreddychem@gmail.com

Chromatographic Conditions: The HPLC system was operated isocratically with the C_{18} column (150 x 4.6 mm; 5 µ) using a mobile phase composition of acetonitrile: 10 Mm ammonium acetate (pH adjusted to 7.2 with 1% triethylamine) in the ratio of 60:40% v/v at a flow rate of 1.0 mL/min and the retention time was 2.244 min within a run time at 10 min. The IBD was detected and quantified at 285 nm.

Method Validation: The method was validated as per ICH Q2(R1) guidelines[13]. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ), precision, reproducibility and robustness.

Linearity: An accurately weighed ivabradine oxalate (equivalent to 10 mg of IBD) was transferred into 10 mL volumetric flasks containing 5 mL of diluent (water; HPLC grade), sonicated to dissolve the working standard and the remaining volume was made up to the mark with diluent to get concentration of 1000 µg/mL. From the working standard solutions (10, 15, 20, 25, 30, 35, 40, 45, and 50 µL) were transferred into a series of 1.0 mL vials and the remaining volume was made up to the mark with water to get final concentrations of 10-50 µg/mL. Aliquots (10 µL) of every solution were injected into HPLC system in triplicate. The calibration curve was plotted over the concentration versus peak area and consequently, the regression equation was calculated.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and limit of quantification (LOQ) values were determined by using calibration curve method according to ICH Q2 (R1)[13] recommendations. The LOD (k=3.3) and LOQ (k=10) values were calculated using the following formula:

$$A=k\sigma/S$$

A is LOD or LOQ; σ is the standard deviation of the response;

S is the slope of the calibration curve

Accuracy: It was performed by adding known quantities of each standard drug related to three concentration levels - 50, 100 and 150 % - of the labelled claim to the marketed formulation containing IBD (10 mg). It was calculated as percentage analyte recovered by the developed method.

Precision: Repeatability of injection was studied by injecting ten standard solutions of IBD (40 µg/mL) on the same day and calculate the peak area %RSD values. For each intra- and inter-assay precision, sample solutions of IBD (20, 30 and 40 µg/mL) were injected into HPLC system in triplicate. Reproducibility was performed by different analysts using same instrument as well as same laboratory.

Robustness: It was analyzed by performing the experiments, during which altered the optimized parameters like buffer composition (varied by ± 2.0%) and flow rate (varied by ± 0.1 mL). The retention time, tailing factor and no. of theoretical plates was recorded.

Estimation of Ivabradine in Tablets: Twenty tablets were weighed and crushed to get fine powder in a mortar. An accurately weighed powder equivalent to one tablet was transferred into a 10 mL volumetric flask containing 5 mL of diluent, sonicated to dissolve the powder and the remaining volume was made up to the mark with diluent. The resulting solution was filtered through 0.45 µm nylon membrane filter. An appropriate aliquot of the above solution was pipetted out into a 1.0 mL vial and the remaining volume was made up to the mark with diluent to get final concentrations 30 µg/mL respectively. The solutions (10 µL) were injected into HPLC system in triplicate under optimized chromatographic conditions and calculate the assay values.

3. Results and Discussion

It is required to contemplate the sequent steps involved for the development of chromatographic method. Specifically, the issue with reference to the standardization of sample preparations, choice of mobile phase, selection of stationary phase and choice of detector has to be emphasized. The optimized chromatographic conditions were selected based on sensitivity, retention time, baseline drift and peak shape. The retention time for IBD at a flow rate of 1.0 mL/min was 2.244 min. The analyte peak was well resolved and free from tailing (Figure 1). The optimized method was accurate for the quantification of IBD, since no interfering peaks appeared close to the retention time of the compound of interest. The tailing factor for IBD was 1.395, thus reflecting good peak symmetry. The no. of theoretical plates for IBD was 3756, so indicating good column efficiency (Table 1). From the specificity studies, it was confirmed that no interference was observed from placebo or individual analyte and also peak purity results were within the acceptance criteria. Therefore, it was proved that the developed method was extremely specific with respect to the placebo and analyte.

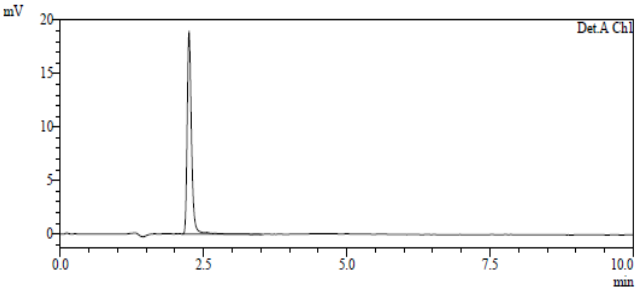

Figure 1: A Standard Chromatogram of IBD

Table 1: Results of System Suitability Parameters

Parameter*	APR	Limit
Peak Area (%RSD)	1.4	NMT: 2 %
Retention Time (%RSD)	0.44	NMT: 1 %
Tailing Factor	1.4	NMT: 1.5
No. of Theoretical Plates	3756	NLT: 2000

** Replicates of six determinations; RSD: Relative Standard Deviation; NMT: Not More Then; NLT: Not Less Then*

The linearity curve was constructed by plotting calibration curve between concentrations versus peak area, showed linear in the concentration range of 10-50 µg/mL for IBD (Figure 2). The regression coefficient values of IBD (R^2 = 0.999) signify that a decent linear relationship exhibited between concentration versus peak area over a wide range (Table 2).

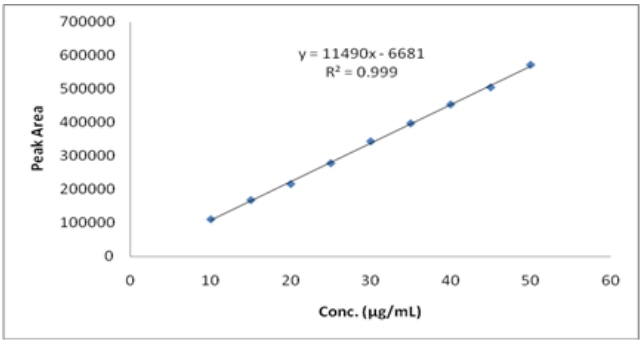

Figure 2: Linearity curve of IBD

The LOD value of IBD was 0.0036 µg/mL whereas LOQ value was found to 0.0109 µg/mL. The obtained results indicate that the developed method was more sensitive. The recovery data has been confirmed that the percentage recovery was within the range (*i.e*. 98 to 102 %) and additionally %RSD values were also below 2.0%. Hence, the results indicate that the developed method was more accurate (Table 3).

Table 2: Linearity Data of IBD

Conc. (µg/mL)	Peak Area (Mean ± SD) *	RSD (%)	Linear regression equation
10	110684 ± 1060	0.957	y = 11490x – 6681 R^2 = 0.999
15	167914 ± 717	0.427	
20	215890 ± 3353	1.553	
25	278088 ± 998	0.359	
30	343236 ± 2649	0.772	
35	396953 ± 967	0.244	
40	453369 ± 1064	0.235	
45	504450 ± 5051	1.001	
50	571573 ± 4871	0.852	

*Replicates of three injections; SD: Standard Deviation; RSD: Relative Standard Deviation

Table 3: Results of Recovery Study by Standard Addition Method

Amount of STD spiked		Amount of sample taken (mg)	% Recovery (Mean ± SD)	RSD (%)
%	mg			
50	5	10	100.44 ± 1.239	1.233
100	10	10	99.60 ± 0.503	0.505
150	15	10	99.87 ± 0.684	0.685

*Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard deviation; SEM: Standard Error of Mean

Injection repeatability value (%RSD) of IBD was found to be 1.034. The intra- and inter-assay precision results were expressed as %RSD values and were shown in (table 4). The low %RSD values proved that the method was precise. The reproducibility results were observed that there were no significant difference between %RSD values obtained (Table 4), which indicates that the developed method was reproducible. There were no significant changes in the retention time, tailing factor and no. of theoretical plates of IBD when the flow rate and buffer composition were changed, which indicates that the developed method was robust (Table 5 & 6).

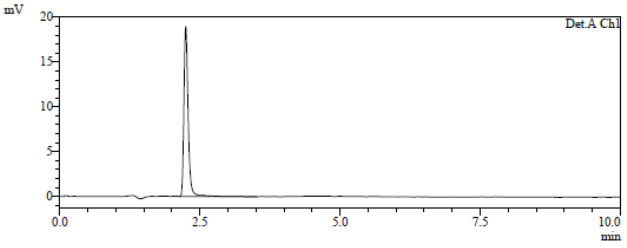

Figure 3: A Sample Chromatogram of IBD

The assay results (Table 7) were proved that the developed method was selective for the estimation of ivabradine without interference of the excipients, which were present in the tablet dosage form (Figure 3).

Table 5: Results for Robustness Study of the Developed HPLC Method by Change of Buffer composition

Parameter	Used	Retention time	Tailing Factor	No. of Theoretical Plates
		Mean± SD*	Mean± SD*	Mean± SD*
Buffer composition %v/v	38	2.235±0.003	1.476±0.004	3575±12.767
	40	2.29±0.009	1.435±0.005	3738±7.024
	42	2.308±0.006	1.493±0.006	55391±111.27

**Replicates of three determinations; SD: Standard Deviation*

Table 6: Results for Robustness Study of the Developed HPLC Method by Change of Flow Rate

Parameter	Used	Retention Time	Tailing Factor	No. of Theoretical Plates
Flow rate (mL/min)	0.9	2.518±0.024	1.489±0.014	4242± 39.154
	1	2.294±0.003	1.433±0.006	3776±20.793
	1.1	2.063±0.006	1.471±0.002	3273±5.568

**Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard Deviation;*

Table 7: Assay Results for Ivabradine in Tablet Dosage Form

Brand Name	Analyte	Label claim (mg)	% Analyte Estimated (Mean ±SD)*	RSD (%)
Ivabrad	Ivabradine	10	100.19± 0.777	0.776

**Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard Deviation;*

4. Conclusion

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of IBD from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures and low retention time. All these factors made this method suitable for quantification of IBD in tablet dosage forms. The method can be successfully used for routine analysis of IBD in bulk drugs and pharmaceutical dosage forms without interference.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

References

1. KD Tripathi. Essentials of Medical Pharmacology, 7th Edi. Jaypee Brothers Medical Publishers Pvt. Ltd., New Delhi, India 2013.
2. Ferrari R, Pavasini R, Camici PG, Crea F, Danchin N, *Pinto* F, Manolis A, Marzilli M, Rosano GMC, Lopez-Sendon J and Fox K. Anti-anginal drugs–beliefs and evidence: systematic review

Table 4: Intra-, Inter-day Assay Precision and Reproducibility Data of the Proposed Method

Analyte IVD Conc. (µg/mL)	Assay Precision				Reproducibility*			
	Intra-day		Inter day		Analyst One		Analyst Two	
	*Mean±SD	%RSD	Mean±SD	%RSD	*Mean±SD	%RSD	*Mean±SD	%RSD
20	100.44±0.800	0.796	100.53±1.105	1.099	100.56±1.104	1.089	100.71±0.917	0.911
30	100.51±0.711	0.708	100.84±1.031	1.022	101.16±0.711	0.702	99.52±1.385	1.392
40	99.99±1.589	1.589	99.66±1.011	1.015	99.92±0.349	0.35	100.53±0.898	0.894

*Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard deviation; SEM: Standard Error of Mean

covering 50 years of medical treatment. Eur Heart J 2019; 40(2): 190–194.
3. Daniel A *Jones*, Adam Timmis and Andrew Wragg. Novel drugs for treating angina. Br Med J 2013; 347: f4726.
4. Ivabradine. https://www.drugbank.ca/drugs/DB09083 (Assessed on 16 December 2018)
5. Md. Rezowanur Rahman, Md. Asaduzzaman and Ashraf Islam. Development and validation of RP-HPLC method for analysis of ivabradine hydrochloride in tablet dosage forms. Res J Pharm Biol Chem Sci. 2011; 3(3): 1032-1043
6. B. P. Srinivasan and Sunitha Seerapu. Development and Validation of RP-HPLC Method for the Estimation of Ivabradine Hydrochloride in Tablets. Ind J Pharm Sci 2010; 72(5): 667–671.
7. Selva Kumar P., Pandiyan K., Rajagopal K. Development and validation of stability indicating rapid HPLC method for the estimation of Ivabradine Hydrochloride in solid oral dosage forms. Int J Pharm Pharm Sci 2014; 6(4): 378-382.
8. Sagarika Panda and Srikanta Patra. Rapid and selective UV spectrophotometric and RP-HPLC methods for dissolution studies of ivabradine controlled-release formulations. PharmaTutor 2014; 2(8); 201-213.
9. Shweta Maheshwari, Amit P. Khandar and Anurekha Jain. Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form. Eur J Anal Chem 2010; 5(1): 53–62.
10. Prajakta Gopinath Thete, Ravindranath Bhanudas Saudagar. Analytical Method Development and Validation for the Determination of Ivabradine HCl by RP-HPLC in bulk and Pharmaceutical Dosage form. Asian J Pharm Tech 2019; 9(2): 89-92.
11. Pooja A. Patil, Hasumati A. Raj, Gautam B. Sonara. Q-Absorbance Ratio Spectrophotometric method for simultaneous determination of Atenolol and Ivabradine HCl in synthetic mixture. Asian J Res Pharm Sci 2016; 6(1): 27-33.
12. Pooja A. Patil, Dr. Hasumati A. Raj and Dr. Gautam B. Sonara. Simultaneous Estimation of Atenolol and Ivabradine HCl using UV-Spectrophotometry. Asian J Pharm Anal 2016; 6(2): 109-114.
13. International Conference on Hormonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure: Text and Methodology {ICH- Q2 (R1)}; November 2015: 1-13.