IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

A Review on Stability indicating RP-HPLC method development and validation for estimation of IVABRADINE in bulk and pharmaceutical dosage form.

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Abstract :

The rising incidence of chronic heart failure and the growing challenge of adult heart failure problem have underscored the need for accurate, reliable and rapid analytical techniques to ensure the quality or therapeutic efficacy of adults who have chronic heart failure medications called hyperpolarizationsdf channel blockers. Stability indicating , High- performance liquid chromatography (HPLC) , particularly reverse phase HPLC (RP-HPLC) , remains a crucial analytical tool for pharmaceutical analysis due to its precision, accuracy, limit of quantitation , limit of detection, sensitivity, and reproducibility . This study aims to stability indicating RP-HPLC method development and validation for estimation of Ivabradine in bulk and pharmaceutical dosage form with a simple , specific , and robust RP-HPLC method for the quantification of a selected Ivabradine in both its pure and formulated products.

Stability indicating and method development involved systematic optimization of various chromatographic parameters, including the safety , efficacy and quality and the selection of stationary phase , mobile phase composition ,detection wavelength , flow rate . A C18 coloumn in reversed phase was employed , and an isocratic elution using a mixture of Acetonitrile : Phosphate buffer (65:35) pH 4 as mobile phase provided well-resolved, sharp peaks with acceptable retention times. Optimal flow rate and detection wavelength were selected to enhance sensitivity and minimize peak tailing, ensuring accurate and reliable quantification.

Keywords

Ivabradine (IVA) RP-HPLC; validation, stability indicating, forced degradation ; ICH uidelines.

Introduction :

The requirement of stability testing data has been recommended by the USFDA and ICH guidance to understand the influence of various environmental factors on the quality of a drug substance and drug product with time. Selection of suitable formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation, depends upon the stability of molecule. Validation is the process of ensuring that a test procedure performs within acceptable standards of reliability, accuracy and precision for its intended purpose. The validation protocols usually followed is defined by the International Conference on Harmonization (ICH) under their validation for analytical procedures methodology. A "stability-indicating method" (SIM) is (found in the FDA guidance document) defined as "Validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. Linearity may be demonstrated directly on the active substance by linear dilution. The correlation coefficient, Y-intercept, Slope of the regression line and residual sum of square shall be submitted by appropriate statistical method.

Precision reflects the closeness of agreement of a series of measurements between the series measurement obtained from multiple sampling from the same sample under the same condition at the same time. Precision may be considered in three levels repeatability, intermediate precision and reproducibility. . It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 80, 100, and 120% of the standard and analyzed. Recovery (%) and RSD (%) were calculated for each concentration. Sensitivity , evaluated through determination of the limit of detection (LOD) and limit of quantification (LOQ), indicated that the method is capable of detecting and quantifying very low concentrations of the antibacterial agent.

Force degradation studies are undertaken to elucidate inherent stability characteristics. Such testing is part of the development strategy and is normally carried out under more severe condition than those used for accelerated stability studies. Force degradation of the drug substance can help identify the likely degradation products.

The ICH guidelines Q1A(R2) also emphasizes that the testing of these features, which are susceptible to change during storage and are likely to influence quality, safety, and or efficacy, must be done by stability – indicating testing methods. The ICH guidelines Q3B entitled impurities in new drug products emphasizes on providing documented evidence that analytical procedure are validated and suitable for the detection and quantification of degradation products. The ICH guidelines Q6A, which provides notes for guidance on specification, also mention the requirement of stability indicating assays under universal test criteria for both the drug substances and products. ICH guidelines on good manufacturing for active pharmaceutical ingredients (Q7A), which under adoption by WHO, also clearly mentions that the test procedure used in stability testing should be validated and be stability indicating.

A limited validation is carried out to support an investigational new drug (IND) application and a more extensive validation for new drug application (NDA) and marketing authorization

application (MAA). Typical parameters recommended by ICH are as follow (ICH guideline 2005). Specificity (Selectivity) typically these might include impurities, degradants, matrix etc. For clinical and before registration batch of the drug product, the analytical method must demonstrate specificity including degradation study.

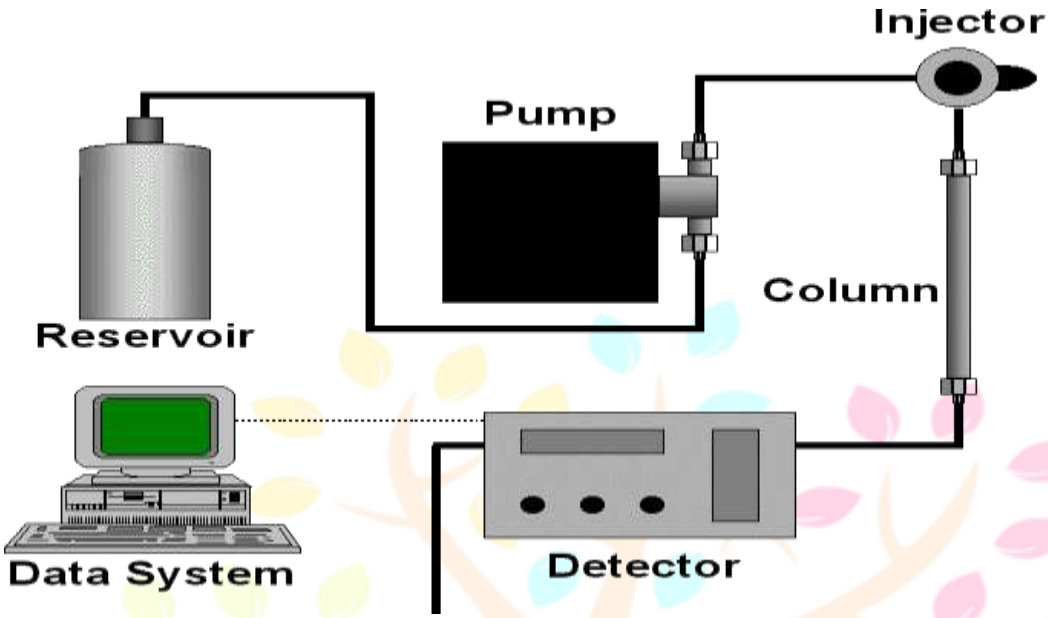

Figure 02: Components of HPLC System

In conclusion the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Ivabradine (IVA) in their pharmaceutical dosage tablet formulations. The method shows good reproducibility, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of tablet dosage formulation of Ivabradine (IVA) can also be successfully performed. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The forced degradation studies were carried out as per ICH guidelines and the results revealed suitability of the method to study stability of IVA under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. it was also concluded that the method is simple, sensitive and ability to separate the drug from degradation products and excipients found in the dosage form. No interference of additives, matrix etc. is encountered in these methods.

Material and Method :

Materials

Active Pharmaceutical Ingredient (API)

The hyper polarization nucleotidegated channel blockers agent selected for analysis was obtained from certified commercial supplier in its pure form. The identify and purity of the API were confirmed using HPLC, UV-Vis Spectrophotometry and NMR spectroscopy, where applicable. All reagents and chemicals used were of AR grade and HPLC grade.

Reagents and Solvents

The solvents used in the mobile phase were Acetonitrile (HPLC grade, obtained from Merck), Methanol (HPLC grade, obtained from Merck), Ortho-phosphoric acid (99% Merck) was used to adjust pH of the mobile phase. All other chemicals and solvents including buffers and standards, were of analytical grade and HPLC grade and used without further purification.

Instruments and Equipment

High-Performance Liquid Chromatography (HPLC)

The analysis was performed using a high-performance liquid chromatography system (waters India) equipped with a photodiode array (PDA) detector and a C18 reversed phase column (4.6 mm × 250 mm, 5µm particle size). The instrument was controlled using ChemStation software for data acquisition and analysis.

UV-Vis Spectrophotometer

A UV-Vis spectrophotometer (Shimadzu UV – 1800), double beam carry – UV-1800 was used for the wavelength scanning of the hyperpolarization- activated cyclic nucleotidegated (HCN) and for determining the optimal detection wavelength for the HPLC method.

Other Laboratory Equipment

pH meter (Eq – 614 A)

Analytical column (C – 18, 4.6 x 150 mm) Balance (CY 104 micro-analytical balance) Ultrasonicator (1.5L 50)

Method Development

Selection of Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout method.

Column : Inertsil 4.6 (id) x 250 mm

Particle size packing : 5 μm

Stationary phases : C18 Inertsil

Mobile phase : Acetonitrile: Phosphate Buffer 10 mm (65:35) pH 4 Detection wavelength

: 285 nm

Flow rate : 1 ml/min.

Temperature : Ambient

Sample size : 20 μL

Mobile Phase Composition

Various mobile phase compositions were evaluated including combination of acetonitrile, water, phosphate buffer solution. A mobile phase of acetonitrile and phosphate buffer (65:35 v/v) was selected, as it provided the best resolution, sharpness, and symmetry of peaks. The pH of the aqueous phase was adjusted to 4.0 using orthophosphoric acid to improve the retention time and peak shape of the analyte.

Flow Rate and column Temperature

The flow rate was optimize 1ml / minute, which ensure a reasonable analysis time while maintaining adequate resolution. The column was maintained at ambient temperature (25^0 C.) throughout the study, as variations in temperature did not significantly impact method performance.

Detection Wavelength

The UV absorption spectrum of the hyperpolarization - activated cyclic nucleotidegated (Ivabradine) was recorded in the range of wavelength 400 to 200 nm using a UV visible spectrophotometer. The corresponding wavelength to the maximum absorbance of the drug (285 nm) was selected for HPLC analysis to ensure high sensitivity and minimal interference from the solvent.

Sample Preparation

Preparation Of Standard Solution

Accurately weighed quantity 10 mg of IVA was dissolved in methanol and volume was made up to 100 ml mark (100 μg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations.

Preparation of standard drug solution

Accurately weighed quantity 10 mg of IVA was dissolved in mobile phase and volume was made up to 100 ml mark. The stock standard solution was diluted further with mobile phase to get final concentration of about 10 μg/ml of IVA.

Method Validation

The developed method validated according to the guidelines set by the ICH for pharmaceutical validation (ICH Q1 (R2).

Accuracy

Accuracy was ascertained on the basis of recovery studies performed by standard addition method. Known amounts of the Ivabradine were added to the placebo matrix at three different concentrations (80%, 100%, and 120% of the nominal concentration.) concentration was analysed and recovery percentage was calculated comparing measured concentration to nominal concentration. A recovery range of 99% to 103% was considered acceptable accuracy for your Ivabrabile.

Precision

Precision was evaluated by analysing five replicate injections of the standard on the same day (intra-day precision) and on different days (inter – day precision). The standard deviation (SD) of the peak areas was calculated. A precision of greater than 2% SD was considered acceptable.

Robustness

The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust.

Linearity and Range-

According to USP tablet powder equivalent to 80,90,100,110,120% of label claims was taken and dissolved and diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions was recorded. IVA marked formulation was found to be linear in the range ±20% of the test concentration of the respective drug.

Forced degradation study

Forced degradation studies of different condition shows all degradants were well resolved from main peak also able to quantify the IVA in the presence of excipients as well as degradants proves method is found to be stability indicating. Hence proposed method adopted for routine analysis in bulk and dosage form

Acid, base, peroxide, thermal and photo degradation. The points will be cover or analyse into force degradation study they are Purity – 1 angle, Purity-1 threshold, Purity flag, area, %degradation.

Summary

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multicomponant system without prior separation and even nano quantities can be estimated. This technique was employed in the present investigation for Method development and validation of stability indicating RP- HPLC method for estimation of the antidiabetic agent in the pharmaceutical dosage form. The estimation of Ivabradine (IVA) and development its stability indicating RP-HPLC method in tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied. HPLC with Inertsil 4.6 (id) x 250 mm column and UV detector was used for the study. The standard and sample solution of IVA were prepared in mobile phase. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.During selection and optimization of the mobile phase it was observed that the sharpness of the peak is achieved with increasing the proportion of methanol whereas the increased proportion of aqueous resulted in broadening of the peak. The mobile phase that was found to be most suitable was Acetonitrile: Phosphate Buffer 10 mm (65:35) pH 4 and detection wavelength 285 nm was selected for the evaluation of the chromatogram of both drugs. The selection of the wavelength was based on the λmax obtained by scanning of standard laboratory mixture. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation. The above results clearly indicate that RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their pharmaceutical formulation without prior separation. Validation of these methods was performed as per the ICH guidelines for these following parameters. Accuracy of the proposed method was ascertained from the recovery studies by

standard addition method. Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed ±S.D. <2. Studies shows that there is no interference of peak from the component of matrix. Studies were carried out only for the two different parameters like different time, different day and different analyst. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the ruggedness of the method under varying condition of its performance. The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust. IVA marketed formulation was found to be linear in the range of 80% to 120 % of test concentration with $R^2 \approx 0.9999$ at selected wavelength for RP- HPLC the methods. Same procedure as described in USP was followed. Forced degradation studies of different condition shows all degradants were well resolved from main peak also able to quantify the IVA in the presence of excipients as well as degradants proves method is found to be stability indicating. Hence proposed method adopted for routine analysis in bulk and dosage form.

Conclusion

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Ivabradine (IVA) in their pharmaceutical dosage tablet formulations. The method shows good reproducibility, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of tablet dosage formulation of Ivabradine (IVA) can also be successfully performed No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The forced degradation studies were carried out as per ICH guidelines and the results revealed suitability of the method to study stability of IVA under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. it was also concluded that the method is simple, sensitive and ability to separate the drug from degradation products and excipients found in the dosage form. No interference of additives, matrix etc. is encountered in these methods.

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