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Review Article

NOVEL METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF BIVALIRUDIN HYDROCHLORIDE USING LIQUID CHROMATOGRAPHY

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ABSTRACT
A liquid chromatographic method for the determination of bivalirudin in bulk and finished pharmaceutical dosage forms has been developed and validated. This method is characterized by its selectivity, accuracy, precision, and linearity over the studied concentration range. The calibration curve exhibited a linear relationship between concentrations of 2-20 µg/ml, with a limit of detection (LOD) of 0.5792 µg/ml and a limit of quantification (LOQ) of 1.775 µg/ml. The method's accuracy was confirmed by a mean percentage recovery of 100.7%, and precision was evidenced by repeatability and intra- and inter-day variations with a relative standard deviation (RSD) of less than one percent. The validated method is suitable for quality control and routine analysis of bivalirudin in both bulk and finished dosage forms, ensuring consistent quality and efficacy in pharmaceutical products.

Keywords: Bivalirudin, Liquid Chromatography, Method Validation, Pharmaceutical Analysis, Quality Control.

INTRODUCTION

Analytical methods are essential in establishing quality standards for pharmaceutical products, ensuring their acceptable efficacy and safety. [1] These methods facilitate the analysis of samples representing any batch, allowing for the assumption that a drug meeting these standards will produce the desired therapeutic effects. Various control actions can be implemented to decide whether to release or reject a product. [2] The objective of method validation is to determine a method's characteristics and limitations, as well as to identify factors that might influence its performance. Generally, method validation should confirm that a method performs adequately across the full range of concentrations of the analyte it is designed to analyze. [3] In modern pharmaceutical analysis, particularly within the field of chromatography [4], method validation is often supported by appropriate experimental design. A recent review highlights the different experimental designs used in chromatography, emphasizing the advantages of an experimental design-based approach over traditional methods. [5] This approach allows for the extraction of extensive data and the drawing of significant conclusions from a relatively small number of well-planned experiments. Liquid chromatography (LC) is a chromatographic technique where the mobile phase is a liquid. Despite being overshadowed by the rapid development of gas chromatography (GC) in the 1950s and 1960s, [6] LC has become the dominant form of chromatography, even replacing GC in some traditional applications. [7] LC's prominence in modern

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pharmaceutical analysis underscores its versatility and effectiveness in analyzing complex compounds and impurities. [8] Bivalirudin is a synthetic peptide composed of 20 amino acids with thrombin-specific anticoagulant properties. [9] It binds reversibly to both the catalytic site and the anion-binding exosite on thrombin, inhibiting the formation and activation of fibrin, factor XIIIa, and other coagulation factors. Bivalirudin is primarily used during coronary angioplasty procedures in combination with aspirin for patients with unstable angina. [10] Recently, it has been explored for off-label use in cardiopulmonary bypass, extracorporeal membrane oxygenation (ECMO), and deep venous thrombosis prophylaxis. [11] In contrast to glycosaminoglycan anticoagulants like heparins, which indirectly inhibit thrombin, bivalirudin directly inhibits thrombin within thrombi and the circulation. [12] This study aims to develop a liquid chromatographic analytical method for bivalirudin hydrochloride and validate the method for its intended use. By doing so, the study seeks to establish a reliable and efficient analytical method that meets regulatory standards and ensures the quality and efficacy of bivalirudin hydrochloride in pharmaceutical formulations.

METHODOLOGY

Chemicals Used

Bivalirudin hydrochloride reference standard was procured Sigma-Aldrich, India. The pharmaceutical dosage form of bivalirudin hydrochloride, Angiomax was procured from local pharmacy with label claim 250mg reconstituted powder manufactured by Pfizer. All chemicals used were of analytical grade. Methanol and water both HPLC grade, were from spectrochem (Mumbai, India). Nylon syringe filters 0.45 µm were from Millex-HN (Mumbai, India).

UV-Spectral Analysis of Bivalirudin

The absorption spectra of reference and test solution were carried out in a one cm quartz cells over the range of 200-400 nm.

Preparation of standard stock solution

Stock solution I

Accurately weighed quantity (10 mg) of bivalirudin was dissolved separately in small quantity of distilled water and volume was made up to 10ml with distilled water to get a solution containing 1000µg/ml.

Stock solution II

From the stock solution, 1ml solution was taken and then diluted up to 10ml with same solvent in a volumetric flask and then concentration of this stock solution was 100µg/ml.

Determination of λ max

Most of drugs absorb light, UV wavelength (200-400 nm) since that contains aromatic double bonds. The solution containing 10µg/ ml of bivalirudin was prepared and scanned over the range of 200-400 nm against distilled water as blank using Shimadzu UV1800 double beam UV spectrophotometer.

Preparation of Calibration Curve

From the stock solution I, stock solution II and stock solution III was prepared to give a concentration of 10µg/ml in distilled water. Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml of stock solution were pipette out into 10 ml volumetric flasks. The selected volumetric flasks volumes were made up to the mark with distilled water. These dilutions give 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml concentration of bivalirudin respectively. The absorbance was measured at 276 nm using UV spectrophotometer [13]

Chromatographic separation

Analytes were separated on an Agilent XDB-C18, 150 x 4.6 mm, 5µm column using an isocratic elution mode. The mobile phase composition consisted of 20 mM potassium dihydrogen phosphate buffer (pH 4.0): acetonitrile (65:35 %v/v). Detection was carried out at a wavelength of 225 nm. A 20 µL fixed-loop was used for the injection of the samples with the flow rate of 1.0 mL min−1. [14]

Preparation of standard solutions

Bivalirudin 50mg weighed accurately and dissolved in methanol to obtain 500µg/ml of bivalirudin standard stock solution. Working standard solution of bivalirudin 50µg/ml was prepared from the stock solution.

Method Validation

The method was validated for the following parameters: linearity, precision, accuracy, selectivity, robustness, limit of quantitation (LOQ), limit of detection (LOD) and system suitability.

Recovery study

To analyze the accuracy of developed method and it was applied to analyze commercially available bivalirudin for injection 250mg by Pfizer pharmaceuticals. Weighed 100mg of bivalirudin for injection and transferred to the 100ml volumetric flask. Then 10ml of distilled water as a solvent was added and kept for 15-20 min with frequent shaking and volume made up to the mark with solvent. Then solution was filtered through Whattman filter paper and this filtrate suitably diluted with distilled water as a solvent to get the solution of 06µg/ml concentration. Then sample absorbance was measured against the blank solution and

recovery was performed at three different levels which was 80%, 100% and 120%. Pre-analyzed sample solution, a known amount of standard drug solution was added at three different levels and absorbances were recorded.

RESULTS

UV-Spectral Analysis of Bivalirudin

With the help of a UV spectrophotometer and with dilutions of medication (10µg/ml) in distilled water, the absorbance of bivalirudin in the UV range of 200-400 was determined. At 276nm, the maximum absorbance was determined and thus the absorption maximum of the drug was determined. The results were shown in figure 1

Preparation of calibration curve

To determine the calibration curve for Bivalirudin drug, 100mg of the pure drug were weighed accurately and made up to 100ml in water, which was Stock-A. 10ml of Stock-A was then taken out of Stock-A and made up to 100ml in distilled water, which was Stock-B. There are several dilutions that have been made using this method, including 2, 4, 6, 8, 10, 12,14,16,18 and 20µg/ml. The regression values were also calculated to be 0.991, and the calibration values have been shown in table 1 as well as the image has been shown in figure 2

Chromatogram of Bivalirudin

Sample solution was analysed on Agilent XDB-C18, 150 x 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 20 mM potassium dihydrogen phosphate buffer (pH 4.0): acetonitrile (65:35 %v/v). Sample was detected at 254 nm. 20µL fixed loop injector was used for the injection of the samples with the flow rate of 1.0 mL min−1. The results on the chromatogram of bivalirudin shown in the figure 3

Method Validation

The method was validated as per ICH guidelines with respect to parameters defining linearity, precision, accuracy, LOD&LOQ, Repeatability and Recovery study. The number of theoretical plates, peak tailing and resolution factor were determined to define system suitability parameters for bivalirudin hydrochloride. The results for system suitability data are listed in Table 1

Linearity and Range

The linearity of response obtained between 2 to 20µg/ml concentrations and calibration curve were obtained by plotting absorbance versus concentration data and treated by linear regression analysis. The calibration curve equation for bivalirudin is y = 0.0062x and calibration curve was found to be linear in the above mentioned concentration and correlation coefficient (R2) was 0.991. The linearity and range resulted from regression analysis of bivalirudin was found to be 2-20µg/ml.

Precision & Repeatability

Repeatability has been determined by analyzing samples 20µg/ ml of bivalirudin for six times, the results are reported in Table 2.

Precision of the method was studied as intra-day and inter-day variations. An intra-day precision was determined by analyzing 06, 12, 18µg/ml of bivalirudin for three times within a day. An inter-day precision was determined by analyzing same concentration of solutions daily for three days.

Accuracy

The accuracy of the method was determined by recovery studies in the synthetic laboratory mixture. Sample absorbance was measured against the blank solution and recovery was performed at three different levels which were 80%, 100% and 120%. Pre-analyzed sample solution, a known amount of standard drug solution was added at three different levels and absorbance was recorded. The drug content of preparation was calculated using the standard calibration curve and amount of drug estimated. The results for recovery studies of bivalirudin has been shown in below table 4

Limit of detection & limit of quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.5792 µg/ ml and 1.775 µg/ml respectively.

Table 1: System suitability parameters for Bivalirudin Hydrochloride

S.NO	System Suitability Parameters	Sample (Bivalirudin)
1	Theoretical plates per colum	8245
2	Symmetry factor/tailing factor	1.254
3	Resolution factor	7.125

Table 2: Repeatability test for bivalirudin

S.No	Concentration µg/ml	Wavelength nm	Absorbance	Mean±S.D	Percentage R.S.D (%)
1	20	276nm	0.119	0.120±0.0066	0.251150
2			0.121		
3			0.122		
4			0.121		
5			0.119		
6			0.122		

Table 3: Intra-day and inter-day precision of Bivalirudin

Drug	Conc. (µg/ml)	Intra-day mean Absorbance ± S.D.	Percentage RSD (%)	Inter-day Mean Absorbance ± S.D.	Percentage RSD (%)
Bivalirudin	06	0.037±0.0004	0.127	0.038±0.0009	0.247
	12	0.071±0.0003	0.491	0.073±0.0001	0.241
	18	0.117±0.0004	0.375	0.115±0.003	0.280
Mean Percentage RSD (%)			0.331		0.256

Table 4: Accuracy data of bivalirudin

Drug	Amount of Drug (µg/ml)	Amount of Drug (µg/ml)	Amount of Drug added (µg/ml)	% Recovery	Average % Recovery
Bivalirudin	20	**80**	**18**	**100.9**	**100.7**
	20	**100**	**20**	**101.4**	
	20	**120**	**22**	**99.9**	

Figure: 1 Determination of λ max of bivalirudin

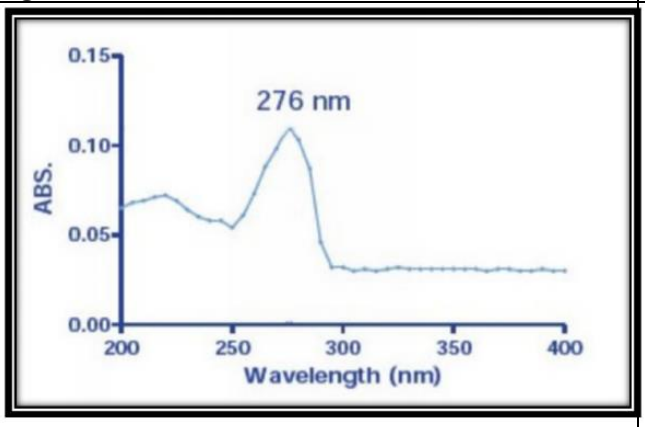


Figure: 2 Calibration curve of Bivalirudin

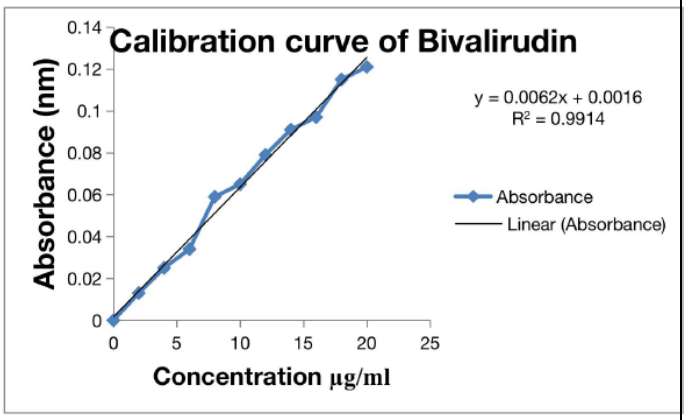
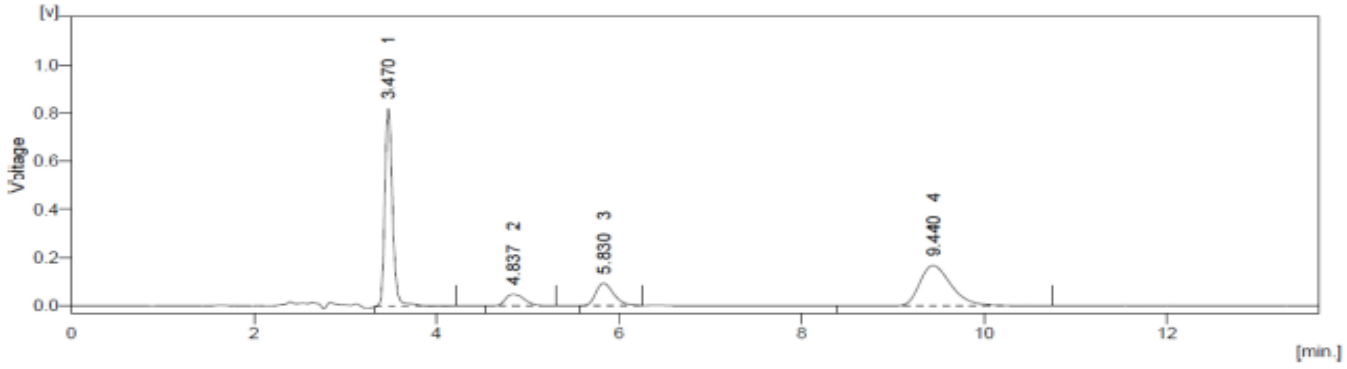


Figure 3: Chromatogram of Bivalirudin hydrochloride in 20mm Potassium dihydrogen phosphate buffer (pH-4): Acetonitrile (65:35%V/V) with flow rate-1.0ml/min



DISCUSSION

The developed spectrophotometric method for measuring bivalirudin in bulk and finished pharmaceuticals has demonstrated significant potential in terms of sensitivity, accuracy, rapidity, precision, and cost-effectiveness. Utilizing UV spectrophotometric absorption, with maximum absorbance observed at 276 nm, the method proves to be straightforward and efficient. Key validation parameters such as the Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.5792 µg/ml and 1.775 µg/ml, respectively. These low values indicate the method's high sensitivity, allowing for the detection and quantification of even minute amounts of bivalirudin, which is crucial for ensuring the quality and efficacy of pharmaceutical formulations. The calibration curve plotted for bivalirudin at 276 nm exhibited a strong linear relationship within the concentration range of 2-20 µg/ml. This linearity confirms that the method can accurately quantify bivalirudin across a wide range of concentrations, making it versatile for different sample types and conditions. Accuracy was assessed through mean percentage recovery, which was found to be 100.7%. This high recovery rate indicates that the method is not only accurate but also reliable, with minimal deviation from the true value. Precision, another critical aspect, was evaluated by examining repeatability, as well as interday and intraday variations. The % Relative Standard Deviation (RSD) was found to be less than one, demonstrating excellent precision and reproducibility of the method.

CONCLUSION

The developed liquid chromatographic method for bivalirudin in bulk and pharmaceutical finished dosage forms has been successfully validated, demonstrating selectivity, accuracy, precision, and linearity over the studied concentration range. These attributes confirm that the method is robust and reliable, making it well-suited for quality control and routine analysis. The method's applicability extends to the determination of bivalirudin in both bulk and finished pharmaceutical products, ensuring consistent quality and efficacy. Thus, this validated chromatographic method provides a valuable tool for the pharmaceutical industry in maintaining high standards of drug analysis and quality assurance.

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