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AN EXPERIMENTAL DESIGN APPROACH FOR THE SYSTEMATIC EVALUATION OF THE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF BIVALIRUDIN HYDROCHLORIDE

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ABSTRACT

This study aimed to validate a liquid chromatographic technique for bivalirudin hydrochloride. According to the literature review, there are few references to bivalirudin hydrochloride and its contaminants in pharmaceutical dosage forms and UV spectrophotometric determination of bulk and tablet. A spectrophotometric method has been devised to measure bulk and finished bivalirudin medicines. The proposed approach uses UV spectrophotometric absorption as a solvent; UV absorbance is 276 nm. LOD and LQ were 0.5792g/ml and 1.775g/ml. Bivalirudin's calibration curve at 276nm was linear between 2-20g/ml. After calculating mean percentage recovery, the method's accuracy was 100.7%. Repeatability increased precision. Selective, accurate, precise, and linear over the examined concentration range. The proposed approach is useful for quality control, routine analysis, and determination of bivalirudin in bulk and medicinal dose formalities, inter and intraday fluctuations, and RSD 1%.

KEY WORDS: Formulation, Oral, Metaprolol, Nanoemulsion

INTRODUCTION

People in the health care industry exercise skill or judgment, or provide services related to preventing or improving people's health or to treating, caring for, or treating people who are injured, sick, disabled or infirmed. Interdisciplinary teams play an important role in delivering modern health care [1]. Using diagnosis and treatment, the medical model focuses on eradicating illness. The health care system relies heavily on medicines. The health care system relies heavily on medicines. Quantification of any medicine begins at the very beginning of its discovery to ensure its quality and efficacy [2]. Analytical methods are used to assign quality standards to products so that they have acceptable efficacy. All samples representing any batch are analyzed for these standards, so it can be assumed that the drug/medicine having these standards will have the desired effect [3]. There are multiple types of control actions that can be used to determine whether to release or reject a product. The purpose of method validation is to determine the characteristics and limitations of a method as well as identify influences that might change them. As a general rule, validation of a method should include checking that it performs adequately across the whole range of concentrations of the analyte it is intended to analyse [4].

Particularly in the field of chromatography, modern pharmaceutical analysis supports method validation by utilizing appropriate experimental design. One of the latest reviews describes all kinds of experimental designs used in chromatography, which is often discussed in review papers [5]. Robustness testing, a crucial component of method validation, shows the importance of experimental design application. This paper proposes a robustness testing approach for RP-HPLC, a method used to analyze data. The number of papers investigating bivalirudine impurities is extremely limited. The efficacy and safety of a medicinal product can only be assured by analytical monitoring of its quality [6]. Therefore, the overall purity of a medicine must be assessed throughout its storage, distribution and use.

Chromatography

Chromatography is a technique by which a mixture is separated into its components as a result of the relative ability of each component to be eluted along or through the stationary phase by mobile phase.

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The word chromatography is derived from the Greek letter's chromos meaning color and the graph means colour writing. The initial use of the terms is attributed to Tswett, who separated colour bands of plant pigments on a chromatography column that consist of an adsorbent powder that was washed with a liquid solvent termed as mobile phase. This is carried down the length of the tube that contains an immobile solid or liquid phase i.e. stationary phase [7].

Analytical Method

Validation is a process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical testing of a pharmaceutical product is necessary to ensure the purity, stability, safety and efficacy. Analytical method validation is an integral part of the quality control system.

Liquid chromatography (LC)

The technique of LC is much older than GC but was overshadowed by the rapid development GC in the 1950's and 1960's. However, LC is currently the dominate type of chromatography and is even replacing GC in some of GC's more traditional applications

Two main types of partition chromatography based on the type of stationary phase

a) Normal-phase liquid chromatography
b) Reversed-phase liquid chromatography

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

Instrumentation

Instruments used were UV-visible double beam spectrophotometer model Shimadzu UV1800 with one cm matched quartz cells and AJ-Vibra electronic balance manufactured by Essae Teraoka Ltd., Made in Japan. The absorption spectra of reference and test solution were carried out in a one cm quartz cells over the range of 200-400 nm [8].

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 [9].

Preparation of Calibration Curve

Stock solution II and III

From the stock solution I, stock solution II and stock solution III were 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 [10].

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}.

METHOD VALIDATION [11, 12]

System Suitability Test

These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole.

Linearity

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.

Precision

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a

homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation.

LOD & LOQ

Determination of the detection and quantification limits was performed based on the standard deviations of y-intercept and the slope of the least square line parameters as defined in the International Conference on Harmonization (ICH) Q2 guidelines

Repeatability

Repeatability has been determined by analyzing samples 20µg/ ml of bivalirudin for six times

Recovery study

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

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

The regression values were also calculated to be 0.991, and the calibration image has been shown in figure 2.

Chromatogram of Bivalirudin

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

System Suitability Test

The results for system suitability data are listed in Table 2.

Linearity and Range

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 3

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. The results shown in table 4

Accuracy

The results for recovery studies of bivalirudin has been shown in below table 5

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 column	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

R. S. D. = Relative Standard Deviation

Table 4: Accuracy data of bivalirudin

Drug	Amount of drug (µg/ml)	Addition level of drug (%)	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

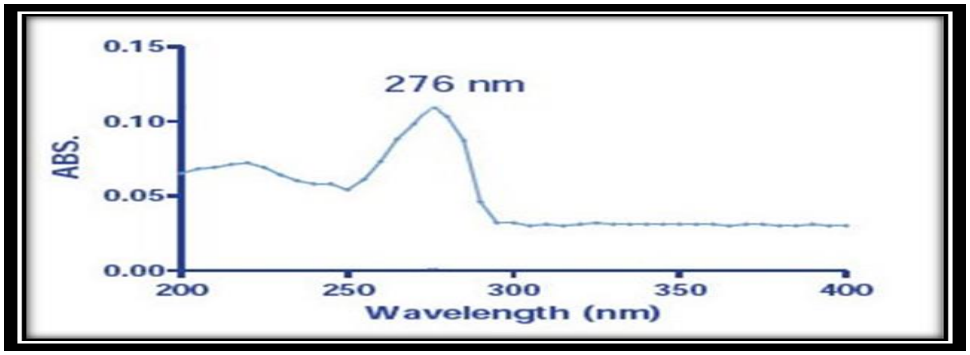


Figure 2: Calibration curve of Bivalirudin

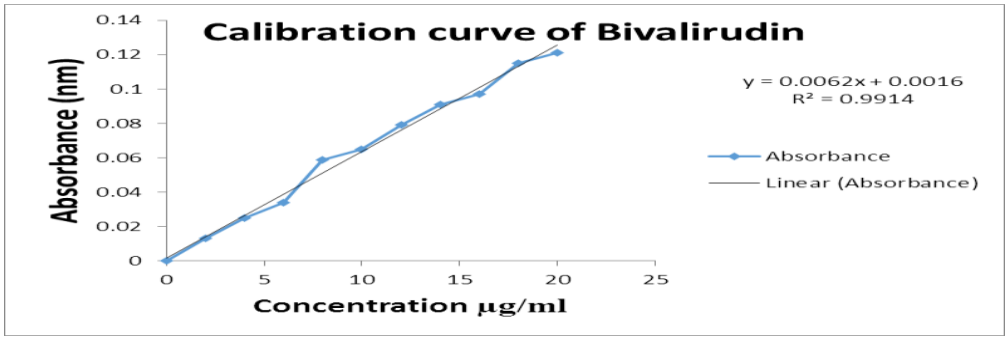
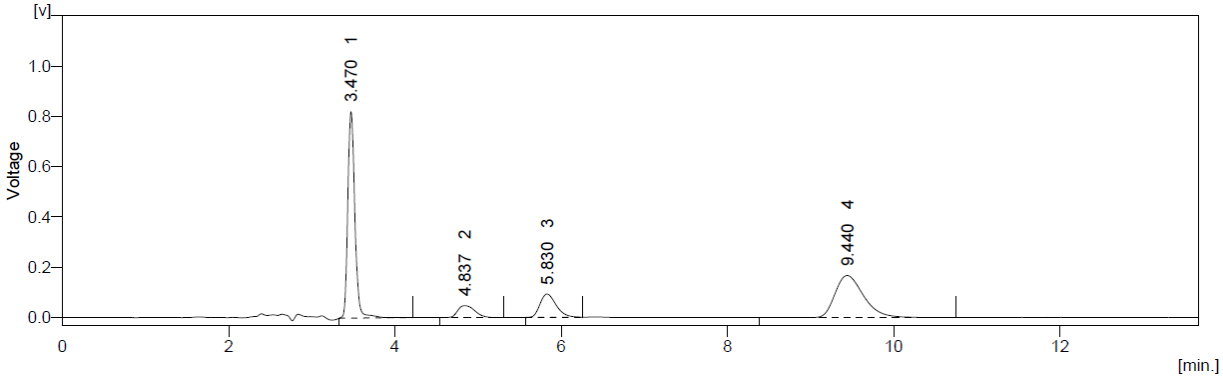


Figure 3: Chromatogram of Bivalirudin hydrochloride in 20mm Potassium dihydrogen phosphate buffer (pH-4): Acetonitrile (65:35%V/V) with flow rate-1.0ml/min



DISCUSSION

In order to develop a sensitive, accurate, rapid, precise, and economical method for measuring bivalirudin in bulk and finished pharmaceuticals, a spectrophotometric method has been developed. UV spectrophotometric absorption is used as a solvent in the proposed method; maximum absorbance is 276 nm in UV region.

Limit of Detection **(**LOD) and Limit of Quantification **(**LOQ) were found to be 0.5792µg/ml and 1.775µg/ml respectively. The calibration curve of bivalirudin plotted at 276nm, and linear relationship was obtained between 2-20µg/ml. Then accuracy of the method was determined by calculating mean percentage recovery it was found to be 100.7%. Further precision was calculated as repeatability, inter and intraday variations and % Relative Standard Deviation (RSD) was less than one.

CONCLUSION

The development and validation of a liquid chromatographic technique for bivalirudin in bulk and pharmaceutical final dosage form. Over the concentration range under study, the suggested approach is linear, accurate, exact, and selective. The suggested approach can be used to determine bivalirudin in bulk and pharmaceutical dosage form, as well as for quality control, routine analysis, and determination.

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