WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 4, 2123-2132. Research Article ISSN 2277– 7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF BRISOPROLOL FUMERATE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROMETRIC METHOD

Swati S. Rawat* and Ganesh Dewan

SND College of Pharmacy, Yeola, Nashik 423401, MS.

Article Received on
18 Feb. 2022,

Revised on 08 March 2022,
Accepted on 28 March 2022

DOI: 10.20959/wjpr20224-23701

***Corresponding Author**
Dr. Swati S. Rawat
SND College of Pharmacy,
Yeola, Nashik 423401, MS.

ABSTRACT

A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of Brisoprolol Fumerate in bulk forms and tablet dosage formulations. The spectrophotometric detection was as per carried out at an absorption maximum of 223 nm using phosphate buffer of pH 6.8 as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for was linear over the selected concentration range 10-50 µg/ml with a correlation coefficient of 0.997. The accuracy was carried out as per recovery study and found between 99.65%% to 100.45%. The results demonstrated that the excipients in the tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of metformin in bulk and formulation.

KEYWORDS: UV Spectroscopy; Method Development; Validation; Brisoprolol Fumerate and ICH Guideline.

1. INTRODUCTION

A spectroscopy method is the branch of science dealing with the study of nteraction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm. Ultraviolet-Visible spectrophotometry[1] UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount

of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer - Lambert law. Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.[2-3]

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law.[4]

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur5. Mathematically, Beer Lambert law is expressed as:

$$A=a\ b\ c$$

Where; A=absorbance or optical density a=absorptivity or extinction coefficient b=path length of radiation through sample (cm) c=concentration of solute in solution. Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A= A.\ 1/1cm.bc$$

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λmax), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal. The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures. They are, use of standard absorptivity value, calibration graph and single- or double-point standardization. In standard absorptive

value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. In calibration graph method, the absorbances of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed.[5]

The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The single point standardization procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.[6-7]

$$Ctest = (Atest \times Cstd)/Astd$$

Where Ctest and Cstd are the concentrations in the sample and standard solutions respectively and Atest and Astd are the absorbances of the sample and standard solutions respectively.

For assay of substance/s in multi component samples by spectrophotometer; the following methods are being used routinely, which includes

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method validation
- Validation is concerned with assuring that a measurement process produces valid measurements.
- Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice.
- A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated,

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and Whenever the method is changed and the change is outside the original scope of the method.
- Nowadays, there are several international renowned organisations offering guidelines on method validation and related topics.
- American Society for Testing and Material (ASTM)
- Codex Committee on Methods of Analysis and Sampling (CCMAS)
- European Committee for Normalization (CEN)
- Cooperation on International Traceability in Analytical Chemistry (CITAC)
- European Cooperation for Accreditation (EA)
- Food and Agricultural Organization (FAO)
- United States Food and Drug Administration (FDA)
- International Conference on Harmonization (ICH). ICH Guidelines (ICH Q2R1) for Analytical procedure and validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.

Types of analytical procedures to be validated The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures: -Identification tests; - Quantitative tests for impurities' content; - Limit tests for the control of impurities; - Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.[8-10] Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision

- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Furthermore, revalidation may be necessary in the following circumstances: -Changes in the synthesis of the drug substance; - Changes in the composition of the finished product; - Changes in the analytical procedure. Aim of Present Work This work deals with the validation of the developed method for the assay of metformin from its dosage form (tablets) using phosphate buffer of pH 6.8. Hence, the method can be used for routine quality control analysis and also stability.

The aim and scope of the proposed work are as under: 1] To develop suitable spectrophotometric method for assay of metformin tablet 2] Perform the validation for the method.[11]

Bisoprolol fumarate has a molecular weight of 766.97. It is a white crystalline powder which is approximately equally hydrophilic and lipophilic, and is readily soluble in water, methanol, ethanol, and chloroform. Bisoprolol fumarate is a selective β1-adrenergic receptor blockers. No intrinsic sympathomimetic activity and membrane stabilizing effect. Animal experiments of different models show that affinity for β1-receptor is 11 to 34-fold larger than β2-receptor. The selectivity for β1 receptor is four times of the similar drugs Atenolol. This product plays the role for a long time (24 hours or more), continual application takes good control of symptoms without tolerance phenomenon, with minimal side effects on the respiratory system, no effects on metabolism of fat. it also has a certain degree of block effect for bronchial β2 receptors, but it may only occur at high doses, usually it has no obvious clinical significance.

2. MATERIALS AND METHODS

2.1 Instruments: The analysis was performed by using the analytical balance (Mettler), pH meter (Cyber scan), UV spectrophotometer (UV-Lambda 25, Perkin Elmer equipped with variable wavelength detector and data integration software).

2.2 Reagents and Solutions: Brisoprolol Fumerate, potassium dihydrogen phosphate, sodium hydroxide analytical grade was used in entire research work.

2.3 Preparation of solvent system: 50ml of 0.2M potassium dihydrogen phosphate solution in 200 ml volumetric flask. Add 22.4 ml 0.2M sodium hydroxide solution and then add to water to make up to volume.

2.3.1 Potassium dihydrogen phosphate (KH_2PO_4): 6.8 gm of dipotassium hydrogen phosphate was weighed accurately and transferred into a 1000 ml volumetric flask containing 900 ml of water and mixed well till clear solution obtained. pH of solution was adjusted up to 6.8 by using Sodium hydroxide. Finally volume make up to 1000 ml with water.

2.3.2 Standard stock solution of bisoprolol fumarate: Weigh accurately about 10mg drug dissolving in phosphate buffer 6.8 in 100 ml volumetric flasks and then make up to volume distilled water. Take into different concentration from 10 -50 µg/ml. Observation was recorded tables and calibration curve was prepared by plotting absorbance v/s concentration of Bisoprolol Fumarate.

2.4. Spectral study: The final solution of Brisoprolol Fumerate scanned in UV spectrophotometer over the range 200- 400nm (Figure 1)

2.5 Method of validation[12]: The developed method was validated according to ICH guidelines.

2.6 Linearity and Range: The stock solution of drug i.e. 10-50ug/ml were transferred into 10ml standard flask and make phosphate buffer 6.8. the absorbance of the solution of different concentration was measured 223 nm against 3 different solution.

2.7. Limit of detection (LOD) It was calculated by using following formula,

$$DL = 3.3\alpha/S$$

Where α: standard deviation. S: slop of calibration curve.

2.8. Limit of quantity (LOQ): It was calculated by using following formula,

$$QL = 10\ \alpha/S$$

Where α: standard of deviation. S: slop of calibration curve.

2.9 Precision: To evaluate repeatability of the method, pure drug of solution within working limit was analyses and being six times. Precision of method was also demonstrated by intraday and inter day variation studies. Intraday studies repeated requirement of standard and sample solution are made in day and % RSD were calculated. Inter day studies are repeated measurement of standard and sample solution were made on 3 consecutive days and % RSD were calculated. The RSD % is not less than 2.0 and indicated high precision for proposed method.

2.10 Accuracy and Recovery study

To ensure the accuracy known the amount of pure drug were added to solvent and these samples are reanalyse by the proposed method and % recovery was studied.

3. RESULTS AND DISCUSSION

The methods discuss in the present work provide convenient, precise and accurate way for estimation of Bisoprolol Fumarate.in bulk and pharmaceutical dosage form using phosphate buffer of pH 6.8.

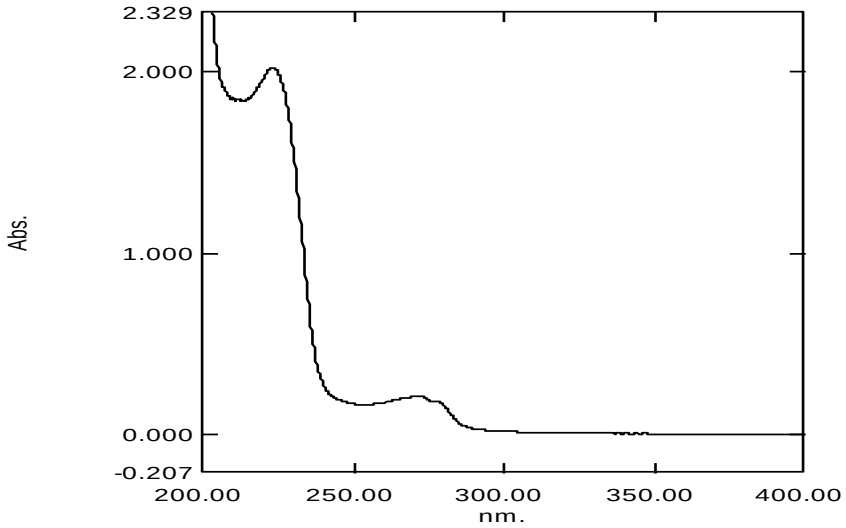

Figure 3.1: UV spectra of bisoprolol fumarate.

The λmax of Bisoprolol Fumarate was found **223 nm** which is nearly same as reported in literature (**225 nm**) and Spectra is shown in Figure.3.1

Table 3.1: Calibration curve in Phosphate buffer 6.8.

Sr.no	Concentration (ug/ml)	Absorbance
1	10(ug/ml)	0.097
2	20(ug/ml)	0.346
3	30(ug/ml)	0.586
4	40(ug/ml)	0.797
5	50(ug/ml)	0.989

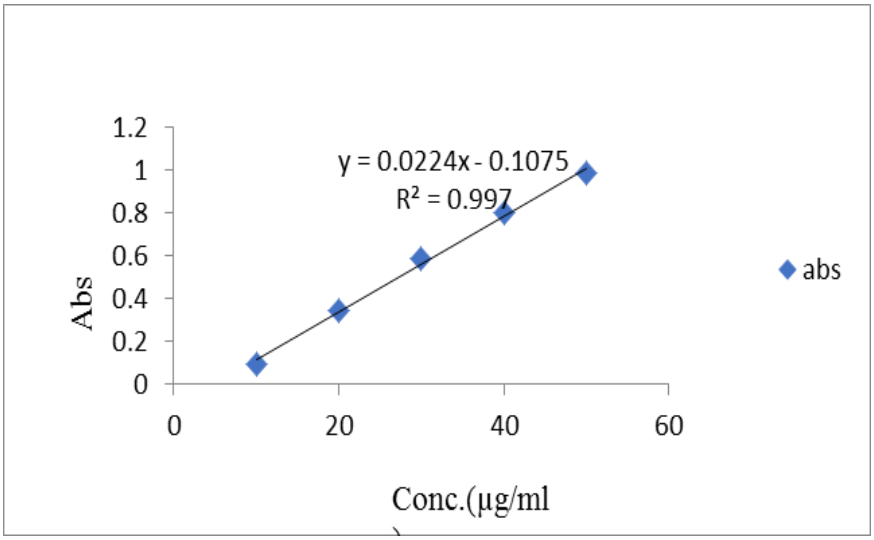

Figure 3.2: Calibration curve of Bisoprolol Fumarate in pH 6.8.

Calibration curve of Bisoprolol Fumarate was carried out at λmax 223 nm in phosphate buffer of pH 6.8. Regression coefficient of Bisoprolol Fumarate was found to be R^2 0.997.the standard linear equation was found to be y= 0.022x-0.107. Graph shown in Figure.3.2

Method of validation

The developed method was validated as per ICH guidelines (ICH Q1B, 1996, ICH Q2 R1, 2005) for following parameters. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions. Repeatability of the method was determined by analysing six samples of same concentrations of drug. Intraday precision was determined by analysing the drugs at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days. Accuracy, LOD, LOQ and Sandell's sensitivity are determined and the results are summarized in Table.3.2

Table 3.2: validation in pH 6.8 phosphate buffer.

Sr. no.	Parameter	Result	ICH Std	Inference
1	Accuracy	100.15%	98%±102%	Pass
2	Precision			
	A.intraday	0.950	RSD<2	Pass
	B.interday	0.950	-	Pass
3	LOD	0.294	-	-
4	LOQ	0.579	-	-

5	Linearity	0.997	>0.997	Pass
6	Range	10-50 ug/ml	-	-
7	STD regression	Y=0.022x-0.107	-	-

4. CONCLUSION

The absorption maximum of Bisoprolol Fumarate was selected at 223nm for the analysis. Regression analysis shows linearity over the concentration range of 10-50µg/ml with correlation coefficient R^2 0.997 (Figure 3.2). The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as percentage recovery. Percentage recovery for was found within the range between 98 % to 100.2%. The % RSD value for was found to be less than 2%. In this study estimation of Bisoprolol Fumarate was carried out by UV spectroscopy method and all the validation parameters found satisfactorily. The result of developed method and validation was given in Table 3.2.

The analytical method for estimation of Bisoprolol Fumarate has been developed and validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for estimation of Bisoprolol Fumarate in bulk as well as formulation.

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