

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BISOPROLOL IN BULK DRUG AND DOSAGE FORM

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1.1 ABSTRACT

Several spectrophotometric and HPLC methods have been reported for determination of Bisoprolol in drugs and pharmaceutical dosage forms. Present study focuses on development of a new, sensitive, suitable and robust reversed-phase high performance liquid chromatography method and validated for the determination of Bisoprolol in bulk drug and in tablet formulation.

In RP-HPLC method, Methanol and Water (75:25 % v/v) was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing UV- detector with Openlab EZchrome software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm. The method gave suitable retention time i.e. 3.60 min for Bisoprolol.

The results of analysis in the method were validated in terms of Filter study, Solution stability, specificity, Linearity, accuracy, precision (Repeatability and intermediate precision), limit of detection, limit of quantification and robustness.

A simple and precise method was developed for the assay of Bisoprolol in bulk drug and in tablet formulation.

1.2 KEYWORDS: RP-HPLC, Bisoprolol, Methanol, Validation.

1.3 INTRODUCTION

In pharmaceutical industries, the validation of analytical method is used to demonstrate that the method is fitted for its purpose; it must follow a plan which includes scopes, Performance characteristics, and acceptance limits. Analytical methods need to be validated or revalidated prior to their introduction into routine analyses. Chromatography is an analytical techniques based on the separation of molecules due to differences in their structure and/or composition. In general, Chromatography involves moving a sample through the system over a stationary phase. The molecules in the samples will have different affinities and interaction with the stationary support, leading to

separation of Molecules. Samples components that display stronger interaction with the stationary phase will move more slowly through the column than components with weaker interaction. Different compounds can be separated from each other as they move through the column. Chromatographic separation can be carried out using a variety of stationary phases. High-Performance liquid chromatography (HPLC) is types of liquid Chromatography used to separate and quantify compounds that have been dissolved in Solution. HPLC can be used to determine the amount of a specific compound in a solution.

1.4. MATERIAL AND METHOD

1.4.1 Preparation of standard stock solution for Chromatographic development:

Bisoprolol Standard stock solution was prepared by dissolving 10.53 mg Bisoprolol (Equivalent to 10 mg of Bisoprolol) into a 20 mL clean and dried volumetric flask, added about 15 mL of methanol to dissolve it completely and made volume up to the mark with methanol (500 PPM).

Further diluted 2 ml of stock solution to 10 mL with mobile phase (100 PPM).

Selection of analytical wavelength for HPLC method development: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 258 nm.

1.4.2 Optimization of HPLC method

Following trials are taken for estimation of Bisoprolol.

Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.

Trial 1:

Chromatographic Conditions:

Standard solution: Bisoprolol 100 PPM

Detector: U.V. Detector

Column: Kromasil C18

Column Dimension: (250 mm X 4.6 mm id.) 5 μ m

Column Oven temperature: 40°C

Injection Volume: 20 μ l

Wavelength: 258 nm

Mobile phase: Methanol: water (70:30)

Flow Rate: 1.0 ml/min

Observation: The trial 1 results are shown in the results.

Trial 2:

Chromatographic Conditions:

Standard solution: Bisoprolol 100 PPM

Detector: U.V. Detector

Column: Kromasil C18

Column Dimension: (250 mm X 4.6 mm i.d.) 5 μ m

Column Oven temperature: 40°C

Injection Volume: 20 μ l

Wavelength: 258 nm

Mobile phase: Methanol: water (75:25)

Flow Rate: 1.0 ml/min

Observation: The trial 2 results are shown in the results.

Optimized Chromatographic condition: Trial no. 2 is considered as optimized chromatography which is as follows

Chromatographic Conditions:

Detector: U.V. Detector

Column: Kromasil C18

Column Dimension: (250 mm X 4.6 mm id.) 5 μ m

Column Oven temperature: 40°C

Injection Volume: 20 μ l

Wavelength: 258 nm

Mobile phase: Methanol: water (75:25)

Flow Rate: 1.0 ml/min

1.4.3 Preparation of System suitability test (Bisoprolol standard solution):

Weighed about 10.53 mg of Bisoprolol (Equivalent to 10 mg of Bisoprolol) and transferred in a 50 mL volumetric flask, added 35 mL of methanol, sonicate to dissolve it, made volume up to the mark with methanol. Pipette out 1.0 ml from standard stock solution and transferred into 20 ml volumetric flask and made volume up to the mark with mobile phase (**Approx 10 μ /mL= working concentration**), chromatograms were recorded.

System suitability is a Pharmacopoeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from Five replicate injection of standard drug solution and the results are recorded.

Acceptance criteria

1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

Analysis of marketed Test sample:

Marketed test sample Having Name Bisoprolol tablets are selected for analysis and for doing validation.

Average weight of test sample (Bisoprolol Tablets):

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula:

$$\text{Average weight (mg)} = \text{Weight of 20 tablets (mg)} / 20$$

Sample preparation of Marketed test sample:

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 50 mg of Bisoprolol and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature

and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further diluted 1.0 ml of filtered stock solution to 50 ml with mobile phase. (10 mcg of Bisoprolol), injected the resultant solution and chromatograms were recorded and results are recorded.

Table No. 6 Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	151.6	100	1	50
Sample 2	151.4	100	1	50

Formula for % Assay calculation:

$$\% \text{ Assay} = \frac{\text{Bisoprolol } Spl \text{ area}}{\text{Bisoprolol } Std \text{ avg area}} \times \frac{\text{Bisoprolol } STD \text{ wt (mg)}}{50} \times \frac{1.0}{20} \times \frac{100}{\text{Tablet sample weight (mg)}}$$

$$\times \frac{50}{1.0} \times \frac{\text{Avg wt of sample (mg)}}{\text{Label claim of Bisoprolol}} \times \text{Factor} \times 100$$

1.4.4 VALIDATION OF RP-HPLC METHOD

The developed method for the estimation of Bisoprolol was validated as per ICH guidelines for the following parameters.

1.4.4.1 FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample.

This study was conducted with Bisoprolol Test sample (Tablet solution).

Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

1.4.4.2 STABILITY OF ANALYTICAL SOLUTION

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions.

The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours.

Standard and Test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial.

1.4.4.3 SPECIFICITY:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Following solution shall be prepared and injected to prove the specificity nature of the method.
(Checked peak purity for standard and test sample solution)

- I. Blank (Mobile phase as a diluent)
- II. Placebo
- III. Bisoprolol Standard solution
- IV. Tablet test sample solution

Analyzing marketed test sample contains excipients (additives) which are totally unknown.

Placebo prepared at lab level by using formula as follows:

Table No. 7 Formula for placebo preparation

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	crospovidone	Disintegrants	5
Total			100 mg

Placebo Sample solution preparation:

Weighed 98.67 mg of placebo material (Which is equivalent to 50 mg of Bisoprolol) and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicate for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable

0.45 μ syringe filter discarding 3-5 mL of initial filtrate. Further dilute 1.0 ml of filtered stock solution to 50 ml with mobile phase, injected the resultant solution and chromatograms were recorded.

1.4.4.4 LINEARITY AND RANGE

Preparation of linearity solution

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

5 levels of Linearity was performed from 10% to 150% of working concentration

Linearity Bisoprolol stock solution:

Weighed 21.05 mg of Bisoprolol (Equivalent to 20 mg of Bisoprolol and dissolved in 50 mL with methanol. Further diluted 5 ml of stock solution to 50 mL with Methanol.

Table No. 8 Linearity levels preparation:

Sr. No.	Level (%)	mL of stock solution	Diluted to with Mobile phase (mL)	Bisoprolol Concentration (μg/mL)
1	10	0.50	20	1.0
2	50	2.50	20	5.0
3	100	5.00	20	10.0
4	125	6.25	20	12.5
5	150	7.50	20	15.0

Determination

Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in μ g/mL on X-axis Vs mean area on y-Axis as given in results.

Acceptance criteria

Correlation Coefficient: NLT 0.98

Intercept: To be report

Slope: To be report

1.4.4.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

1.4.4.6 ACCURACY (% RECOVERY)

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found,

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Refer Following table for each sample:

Table No. 9 Accuracy sample preparation

Level (%)	Bisoprolol Std (mg)	Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Bisoprolol Concentration (µg/mL)
50	26.4	98.6	100	1	50	5.02
	26.3	98.8	100	1	50	5.00
	26.5	98.8	100	1	50	5.04
100	52.7	98.5	100	1	50	10.01
	52.7	98.7	100	1	50	10.01
	52.8	98.5	100	1	50	10.03
150	79.1	98.6	100	1	50	15.03
	79.0	98.8	100	1	50	15.01
	79.2	98.6	100	1	50	15.05

Procedure for preparation of Accuracy sample solution:

Take clean and dried 9 volumetric flask of 100 mL. Weighed approx 98.67 mg of placebo and transferred in each 100 mL volumetric flask. Wighed Bisoprolol API as per accuracy level and transferred in same 100 ml volumetric flask. Add 70-75 ml of Methanol sonicate it for 10 minutes with intermittent shaking. Allowed to cool the solution at room temperature and made the volume up to the mark with Methanol. Filter the solution through suitable 0.45 µ Nylon syringe filter discarding 5 mL of filtrate. Further, dilute 1.0 ml of filtrate to 50 ml with the mobile phase.

Acceptance criteria

1. % Recovery for each sample and Mean recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

1.4.4.7 PRECISION

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test samples.

1.5 RESULT AND DISCUSSION

1.5.1 PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG

1.5.2 Color, odor and appearance

Table No. 12 Color, odor and appearance of Drug

Sr. No	Name	Colour, odor and appearance of drug
1	Bisoprolol	White, odorless and Amorphous powder

1.5.3 Melting point determination

Table No. 13 Melting point of Drug

Sr. No.	Name	Melting point std. value (°C)	Melting point observed (°C)
1	Bisoprolol	190-193 °C	190-192 °C

1.5.4 Solubility study

Table No. 14 Solubility study of Bisoprolol

Sr. No.	Name of Solvent	Observation	Conclusion	Summary
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.	Methanol used as a diluent for preparing stock solution.
2	Methanol	No Drug Particles seen after sonication	Drug was found soluble in methanol.	

Selection of solvent

Methanol was selected as the solvent for dissolving Bisoprolol

Selection of analytical wavelength

1) Blank methanol:

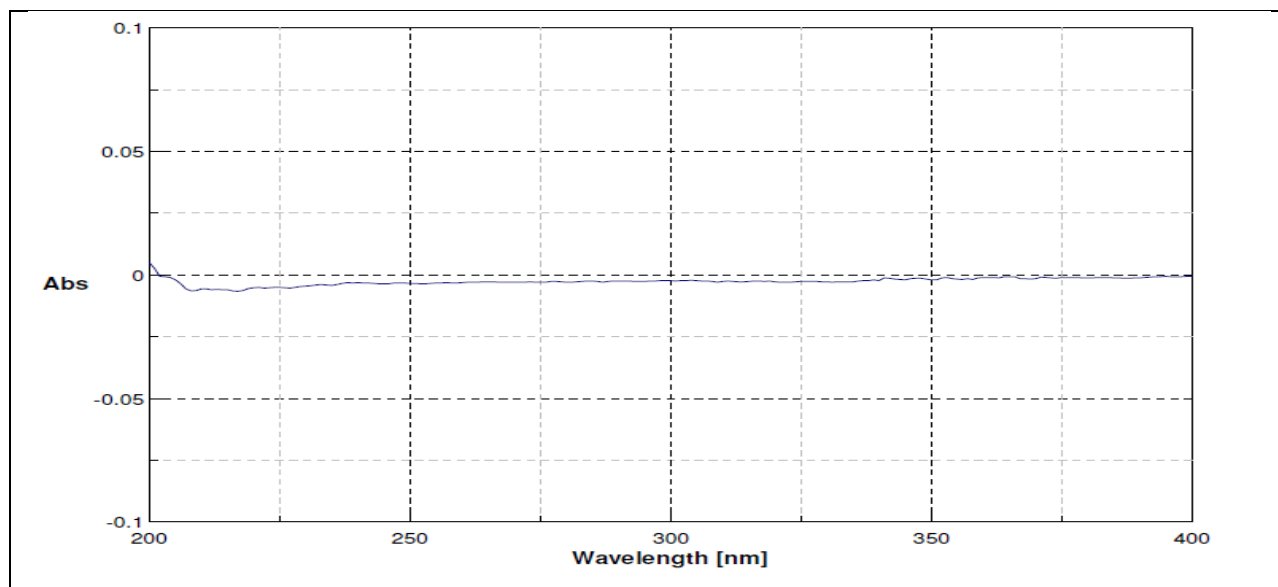


Fig. No. 2 UV spectrum of Methanol as a blank

2) Bisoprolol STD solution: (20 PPM)

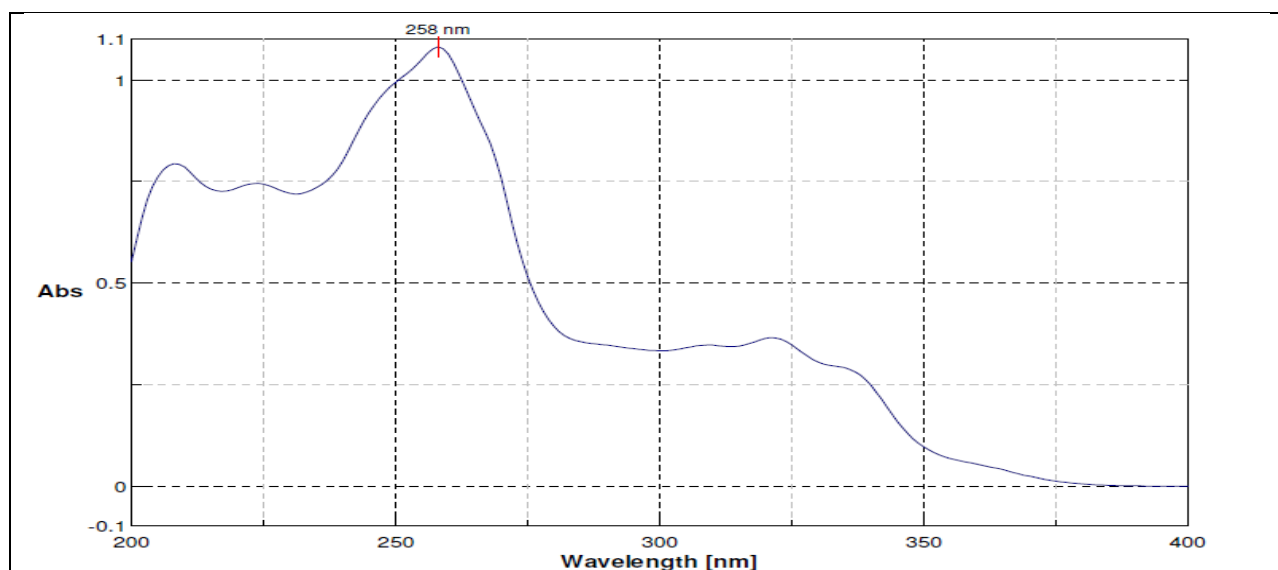


Fig. No. 3 UV spectrum of Bisoprolol

Observation: The standard solution was scanned between 200 nm to 400 nm. Wavelength of maximum absorption was determined for drug. Bisoprolol showed maximum absorbance at 258 nm. It is shown in **Figure No.2**. Therefore 258 nm considered as an analytical wavelength for further determination.

1.6 CONCLUSION

- The present work involved the development of simple, accurate, precise and suitable RP-HPLC method.
- Literature survey revealed that several methods have been reported for determination of Bisoprolol in bulk drug or in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination of Bisoprolol in bulk drug and pharmaceutical dosage form.
- In developed RP-HPLC method, the analyte were resolved by using isocratic program and mobile phase was used Methanol: Water (75:25 % v/v) at a flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector with Openlab EZ-Chrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 μ m. The detection was carried out at 258 nm.
- The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantification.
- The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method.
- Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Bisoprolol and also in pharmaceutical dosage form.

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