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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF BISOPROLOL FUMARATE IN BULK AND TABLET DOSAGE FORM BY UV-SPECTROSCOPIC METHOD

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Abstract:

A Simple, specific, rapid, precise and accurate UV Spectrophotometric method have been developed and Validated for determination of Bisoprolol Fumarate. Drug Bisoprolol Fumarate showed the absorption maxima in at 223.5 nm and was linear for a range of 5 µg/ml –25 µg/ml with correlation coefficient of 0.9992. The validation of the above proposed method was done by carrying out precision and accuracy studies. The percentage recovery at three different levels i.e. 80%, 100% and 120% was found to be 81.5%, 101.15% and 120.7% respectively. The analytical method showed good Intra precision (Repeatability) with relative standard deviation 0.913% and Inter precision with relative standard deviation is 0.568% which is less than 2. The proposed method was validated for the parameter Specificity, Precision, Linearity and range, Ruggedness, Accuracy and recovery. Hence proposed analytical method for estimation of Bisoprolol Fumarate formulation drug in Tablet dosage forms by UV spectrophotometer in pharmaceutical can be applied for the routine quality control analysis.

Keywords: Validation, Bisoprolol Fumarate Tablet, UV Spectrophotometer.

Introduction

Bisoprolol Fumarate is an Anti-hypertensive drug belongs to cardio selective β1 adrenergic blocking agent[1] used in hypertension, stable chronic angina pectoris, stable chronic heart failure and myocardial infarction the IUPAC name is 2-Propanol, 1-[4[[2-(1-Methylethoxy)Ethoxy]Methyl)Phenoxy)-3-[(1-Methylethyl)amino]-,(±)-,(E)-2-Butanediote (2:1) Salt. Bisoprolol Fumarate having molecular formula $(C_{18}H_{31}NO4_4)_2.C_4H_4O_4$ and molecular weight 766.96g/mol. It is official in United States [2] and European/British pharmacopoeia [3] with Chromatographic-Potentiometric titration method. Literature survey reveals that few analytical methods are available including HPTLC [4], HPLC [5-9] and UV Spectrophotometry [10-21].

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In the present work, a simple, accurate and sensitive method for determining Bisoprolol Fumarate content in drug substance pure form and drug product (film coated tablet dosage form) was introduced. No simple and rapid work has been reported for the estimation of Bisoprolol Fumarate (film coated tablet). All these reported methods either took a long time for analysis or employ mobile phases with pH adjustment of Buffer solutions, fine crushing of coated tablet, carefully weighing of crushed tablet powder avoiding coating material for sample preparation, which is tedious and anomalous[4-21], especially for routine testing of quality control samples of assay content study. Hence it was felt necessary to build up a simple, rapid, economical and precise Spectrophotometric method for the direct estimation of Bisoprolol Fumarate. The current research work deals with the development of UV Spectrophotometric method and its validation as per International Conference on Harmonization (ICH) guideline [22-24]. The developed method was found to be simple, specific, stable, rapid, accurate, precise, reliable, less expensive and time saving by UV Spectrophotometric method[10-21] for the estimation of Bisoprolol Fumarate content in drug substance and drug product (Film Coated Tablet Dosage form).

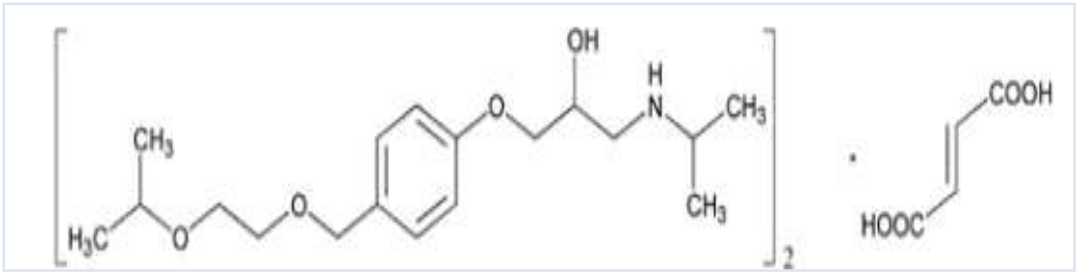

Figure 1: Chemical structure of Bisoprolol Fumarate

Materials and Methods

Instrumentation and Materials:

U.V. visible double beam spectrophotometers SL 210 Elico with Spectra treat software having path length 1cm U.V. matched quartz cells were used. Biselect 2.5 mg Tablet (Bisoprolol Fumarate Film Coated Tablet USP 2.5 mg, Manufacturer - Intas Pharmaceutical) sample procured from market and Standard Bisoprolol Fumarate from Omicron Pharmaceuticals, Surat Gujarat. All chemicals, solvents and reagents i.e. Sodium Hydroxide, Water and Methanol used, were analytical grade and purchased from PCL/Merck Ltd, India, S.D. Fine Chem Ltd/Qualigens.

Method Development:

Preparation of Diluent Solution

Transferred about 700 ml of water to the 1000 ml volumetric flask, then slowly added about 1.0 gm of Sodium Hydroxide with stirring and, mixed well, then with constant stirring slowly added Methanol up to mark to make volume 1000 ml. used this solution as diluent.

Preparation of Standard Solution

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Weighed accurately about 120 mg of Bisoprolol Fumarate and transferred to 200 ml volumetric flask. Dissolved in diluent and made up the volume to 200 ml, further transferred 5 ml of solution to 200 ml volumetric flask. Made volume upto mark to get a concentration 15µg/ml.

Selection of wavelength for analysis of Bisoprolol Fumarate

The standard solution having concentration 15µg/ml was scanned at 200 nm to 400 nm with diluent as the blank to detect maximum wavelength (Figure-2).

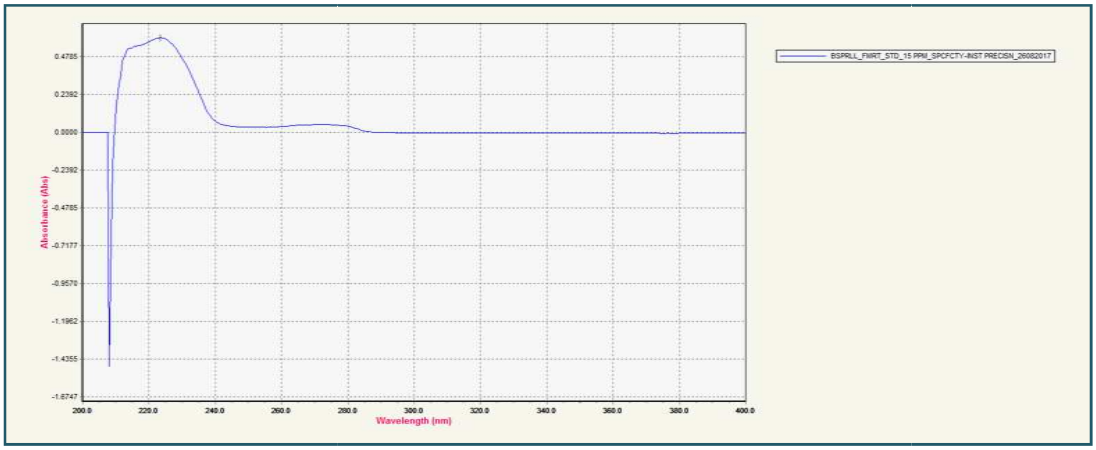

Figure 2: Estimation of Maxima of Bisoprolol Fumarate

From the above (Figure-2) spectra of Bisoprolol Fumarate wavelength maxima identified for quantification were 223.5 nm (λmax).

Validation of proposed Analytical Method

The proposed method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [22-24]. Analysis of variance was used to ensure the validity and performance effectiveness of the proposed analytical methods.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity was done by scanning of Diluent solution and Standard solution of Bisoprolol Fumarate having concentrations 15 µg/ml in Spectrophotometric range from 200 nm to 400 nm to check specific absorption maxima at predefined wavelength i.e. 223.5 nm and solution stability study performed to evaluate the solution stability at different time interval up to 24 hrs.

Instrument Precision

Instrument precision was performed to check the suitability of the developed analytical method with respect to ability of instrument consistency to provide the precise wavelength maxim when scanned the Standard solution of Bisoprolol

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Fumarate having concentrations 15 µg/ml in the UV range from 200 nm to 400 nm. To check specific absorption maxima at predefined wavelength 223.5 nm with reproducible absorption detection. Six separated standard preparations were scanned / analyzed according to the proposed method of analysis. The % RSD due to Bisoprolol Fumarate concentration for the six standards was found 0.214%. The % RSD due to Bisoprolol Fumarate concentration for the instrument precision meets the requirements. Results are tabulated in the Table 1.

Table 1 Instrument Precision

Sr. No.	Standard number	Absorbance @223.5 nm	% RSD
1	Standard Preparation -1	0.5989	0.214% Limit < 2%
2	Standard Preparation -2	0.6007	
3	Standard Preparation -3	0.6014	
4	Standard Preparation -4	0.5984	
5	Standard Preparation -5	0.5994	
6	Standard Preparation -6	0.6013	
Average Absorbance →		0.6000	

Linearity and Range

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug in samples in a given range. Linearity justifies the use of single-point calibrations. The correlation coefficient of the Regression line for was found that 0.9992.

Five levels of five different concentrations Standard solution of Bisoprolol Fumarate having concentrations 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml, in the range relative to the working concentrations, were prepared and read according to the method of analysis. A linear regression curve was constructed, the correlation coefficient (R2) and assessment value calculated. The correlation coefficient (R2) for Bisoprolol Fumarate obtained is 0.9992. The plot is a straight line and the results are tabulated in the Table 2 and Curve is shown in the Figure 3.

Table 2 Linearity and Range

Sr. No.	Standard Concentration (µg/ml)	Absorbance @ 223.5 nm	Correlation coefficient
1	5	0.1493	
2	10	0.3956	
3	15	0.5854	

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4	20	0.7955	0.9992
5	25	1.0285	Limit >0.999

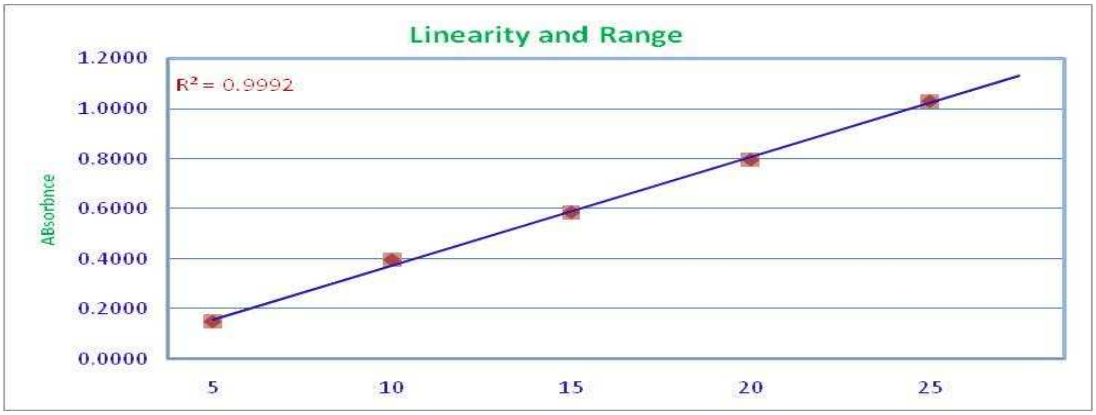

Figure 3: Linearity and Range of Bisoprolol Fumarate

Analytical Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied to multiple sampling of a homogenous sample.

Procedure for analysis of Tablet Formulation: Determined the weight of 10 tablets and transferred to 200 ml volumetric flask. Dissolved in about 150 ml diluent, Sonicated for 20 minute with intermittent shaking and made up the volume to 200 ml with diluent. The solution was filtered through Whatmann filter paper, discarding first few ml of filtrate, and further transferred 3 ml of solution to 25 ml volumetric flask.

Intra Precision (Repeatability)

This parameter determines the repeatability of Bisoprolol Fumarate Tablet USP 2.5 mg assay results under the same operating conditions over a short period of time. The % RSD due to Bisoprolol Fumarate Film Coated Tablet USP 2.5 mg concentration for the six samples was found to be 0.913%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Bisoprolol Fumarate Film Coated Tablet USP 2.5 mg concentration for the assay meets the requirements. Results are tabulated in the Table 3.

Table 3 Intra Precision (Repeatability) Results

Sr. No.	Sample number	Bisoprolol Fumarate Tablet USP 2.5 mg		% RSD of Six Assay content
		% Assay content	Assay content in mg	
1	Sample Preparation -1	101.2	2.530	
2	Sample Preparation -2	100.7	2.517	
3	Sample Preparation -3	100.9	2.522	

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4	Sample Preparation -4	103.1	2.579	0.913%
5	Sample Preparation -5	101.6	2.541	Limit < 2%
6	Sample Preparation -	100.8	2.519	
Average % Assay →		101.4	2.535	

Inter Precision (Repeatability*)*

This parameter determines the Intermediate repeatability of Bisoprolol Fumarate Tablet USP 2.5 mg assay results under the same operating conditions test performed on a different day, using different makes of reagents and solvents. The % RSD due to Bisoprolol Fumarate Tablet USP 2.5 mg concentration for the six samples was found to be 0.568%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Bisoprolol Fumarate Tablet USP 2.5 mg concentration for the assay meets the requirements. Results are tabulated in the Table 4.

Table 4 Inter Precision (Repeatability) Results

Sr. No.	Sample number	Bisoprolol Fumarate Tablet USP 2.5mg		% RSD of Six Assay content
		% Assay content	Assay content in mg	
1	Sample Preparation -1	100.7	2.518	
2	Sample Preparation -2	100.7	2.518	
3	Sample Preparation -3	99.4	2.485	
4	Sample Preparation -4	100.1	2.503	0.568%
5	Sample Preparation -5	100.8	2.520	
6	Sample Preparation -6	99.9	2.497	Limit < 2%
Average % Assay →		100.3	2.507	

Ruggedness

Ruggedness of the method was determined by carrying out the analysis on different days, different makes of reagents and solvents. The respective test assay results of Bisoprolol Fumarate Tablet USP 2.5 mg having concentration as 15µg/ml was illustrious. The result is expressed as shown in table 4. The developed method for estimation of Bisoprolol Fumarate Tablet USP 2.5 mg was found to be rugged as Shown in table 5.

Table 5 Ruggedness

Sr. No.	**Precision**	**% RSD of assay of Six Preparation**	**Limit For Ruggedness**
1	Intra Precision	0.913	

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2	Inter Precision	0.568	NMT 2%
% RSD of Overall 12 Assay content		0.929	

Accuracy

This parameter determines the accuracy of the assay results under the same operating conditions test.

A Bisoprolol Fumarate Tablet USP 2.5 mg sample was constituted analyzed for the accuracy with known quantity of standard samples of Bisoprolol Fumarate at 80%, 100%, 120% concentration levels and assayed as per the method stated under analytical Methods respectively. Three determinations were performed under each concentration levels respectively. Results are shown in Tables 6, 7, 8. The % RSD due to recovery of Bisoprolol Fumarate at 80%,100%, 120% concentration levels was found to be 81.5%, 101.1% and 120.7% respectively. Nine sample preparations were analyzed according to the proposed method of analysis. The %RSD due to Bisoprolol Fumarate Tablet USP 2.5 mg concentration for the assay meets the requirement and accuracy of recovery is within 90.0% to 110%. Results are tabulated in the Table 6, 7, 8.

Table 6 Accuracy and Recovery Results @ 80 % Concentration level

Sr. No.	**Accuracy @ 80% level**	Recovery of Bisoprolol Fumarate Tablet USP 2.5 mg % Assay content	**% Recovery** 90.0% to 110%	**% RSD**
1	Sample Preparation -1	82.0	**101.9**	0.528% Limit< 2%
2	Sample Preparation -2	81.5		
3	Sample Preparation -3	81.3		
Average % Assay →		81.5		

Table 7 Accuracy and Recovery Results @ 100 % Concentration level

Sr. No.	**Accuracy @ 100% level**	Recovery of Bisoprolol Fumarate Tablet USP 2.5 mg % Assay content	**% Recovery** 90.0% to 110%	**% RSD**
1	Sample Preparation -1	101.0	**101.1**	1.322% Limit < 2%
2	Sample Preparation -2	99.8		
3	Sample Preparation -3	102.5		
Average % Assay →		101.1		

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Table 8 Accuracy and Recovery Results @ 120 % Concentration level

Sr. No.	**Accuracy** @ 120% **level**	Recovery of Bisoprolol Fumarate Tablet USP 2.5 mg % Assay content	**% Recovery** 90.0% to 110%	**% RSD**
1	Sample Preparation -1	119.2	**100.6**	1.344% Limit < 2%
2	Sample Preparation -2	120.5		
3	Sample Preparation -3	122.4		
Average % Assay →		120.7		

Solution Stability

Solution stability of the Bisoprolol Fumarate Tablet USP 2.5 mg sample solution was performed up to 26 hrs with different time interval and found the solution is stable showing cumulative % RSD of different time interval is 0.750 which is less than the 2. Hence the Bisoprolol Fumarate Tablet USP 2.5 mg sample solution is found stable up to 24 hrs at room temperature.

Results and Discussion

The method discussed in the present work provides a simple, stable, rapid, accurate, precise, reliable, less expensive (Economical), time *saving* and convenient method for the analysis of Bisoprolol Fumarate Tablet USP 2.5 mg using U.V. Spectrophotometry. λ max selected for quantitation was 223.5 nm. In the developed analytical method, the linearity was observed 0.9992 in the concentration range of 5 µg/ml -25 µg/ml.

Method precision for the Bisoprolol Fumarate Tablet USP 2.5 mg at concentrations level 15µg/ml was found in the range of 99.4%-103.1%. Accuracy of the proposed method was ascertained by recovery studies and the results were expressed as percent recovery and were found in the Range of 100.6%-101.9%.Values of standard deviation and coefficient of variance was satisfactorily indicating the accuracy of both the methods. Intra-day and Inter-day precision studies were carried out by analyzing the sample of Bisoprolol Fumarate Tablet USP 2.5 mg at different time interval on the same day and on different days respectively. Standard deviation and coefficient of variance for Intra-day and Inter-day precision studies was found to be less than 2 indicating precision of the proposed method.

Based on the outcome of analytical method development and analytical validation study test results, it was found that, the proposed analytical method for estimation of Bisoprolol Fumarate and Bisoprolol Fumarate Tablet USP 2.5 mg by UV Spectrophotometry is Accurate, Precise, Reproducible, Stable, Simple, Rapid Time saving and less expensive (Economical). The analytical method can be employed for routine quality control of Bisoprolol Fumarate and

Bisoprolol Fumarate Tablet USP 2.5 mg in pharmaceutical analysis.

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