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QbD APPROACH FOR ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BISOPROLOL FUMARATE BY SPECTROSCOPIC METHOD

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ABSTRACT

An accurate and reliable ultra-violet spectrophotometric method was developed based on the Quality by Design framework, for determination of Bisoprolol Fumarate oral dosage form. According to International Conference on Harmonization (ICH Q8 [R2]) guidelines, an experimental work was planned for both spectroscopic method development and its validation. QbD (Quality-by-Design) approach was implemented for spectroscopic method development and its validation. The research work demonstrated that the UV is valid for the determination of assay of Bisoprolol fumarate. For performing experimental work analytical grade chemical (water, methanol, chloroform, ethanol) was used the spectroscopic method development and validated on UV spectrophotometer by using suitable solvent (water, methanol, chloroform, ethanol) and detection was performed at 223 nm. Target Product Quality Profile (TPQP) and Critical Quality Attributes (CQA). This is helpful to observe the impact of raw materials Critical Material Attributes (CMA), Critical Process Parameter (CPP) on the CQAs. For all the variable parameters as stated in Ishikawa diagram, the absorbance was recorded over the concentration range.

KEY WORDS

QbD, Bisoprolol Fumarate, UV spectroscopic method, TPQP, CQA, CMA, CPP, Ishikawa diagram.

INTRODUCTION

Analytical methods play an important role supporting implementation of QbD in process Pharmaceutical development and development and manufacturing. Analytical testing also plays prominent role in Pharmaceutical development, risk assessment, process monitoring and control and continuous quality assessment throughout the product. Quality-by-Design (QbD) is well-established in development and manufacture of pharmaceutical drug substance and drug product and is discussed in ICH Q8, [1] Q9 and Q2. The same QbD approach can be applied to analytical procedures as per ICH Q2. In addition, there is now a technique to definitively link the data to its intended use. These are exciting times for testing laboratories and the users of the data they produce. The knowledge obtained during development helps to justify the establishment of a design space, (process) control strategy and set point within the (regulatory approved) design space. Materials made within the design space will produce an acceptable product, and changes within the design space are regulatory acceptable. Quality by Design approach suggests looking into the quality of analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into final results of analytical process. QbD is defined as —a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management. In alignment with the approach proposed in the draft FDA guidance for process validation, a three-stage approach can be applied to method validation [2-3].

Bisoprolol fumarate is a synthetic, beta1-selective (cardioselective) adrenoceptor blocking agent. The chemical name for bisoprolol fumarate is (±)-1-[4-[[2-(1-Methylethoxy) ethoxy] methyl] phenoxy]-3[(1-methylethyl) amino]-2- propenol (E)-2-butenedioate (2:1) (salt). It possesses an asymmetric carbon atom in its structure and is provided as a racemic mixture. The S (-) enantiomer is responsible for most of the beta-blocking activity. Bisoprolol fumarate is official in USP [3].
Stage1. Method Design: Define method requirements and conditions and identify critical controls.
Stage2. Method Qualification: Confirm that the method is capable of meeting its design intent.
Stage3.Continued Method Verification: Gain ongoing assurance to ensure that the method remains in a state of control during routine use. A critical function of Stage 1 is the design of an Analytical Target Profile (ATP) for the method. To design the ATP, it is necessary to determine the characteristics that will be indicators of method performance for its intended use. These are selected from the performance characteristics described in ICH Q2 as per the traditional approach. Instead of being applied in a tick box manner,

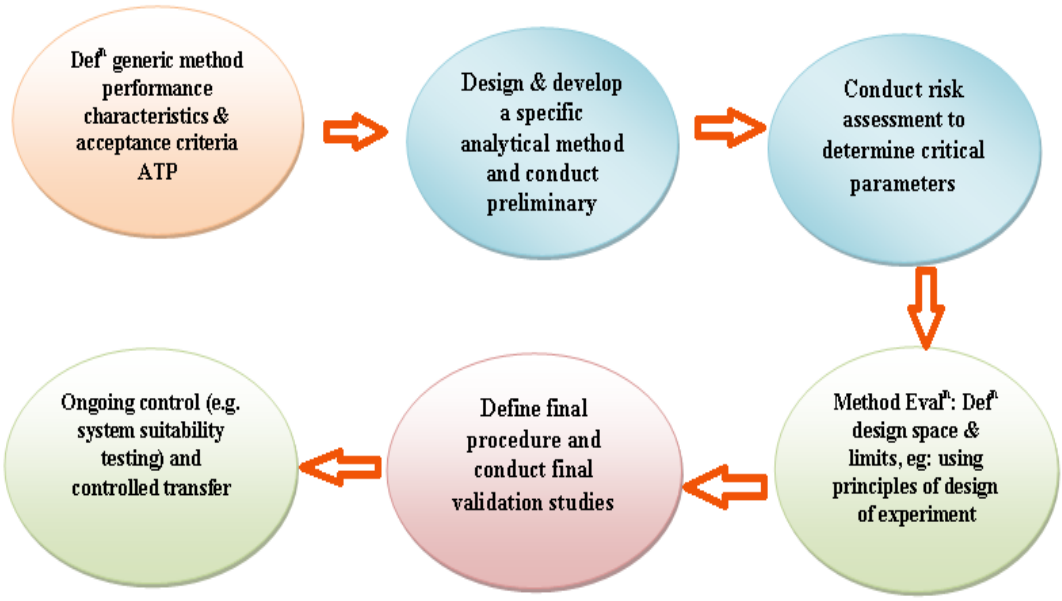

Figure No. 1 QbD workflow

They are Investigated by a risk assessment exercise as described in ICH Q9 in combination with carefully designed development studies to identify the critical method and sources of variation. Variables are then investigated by robustness and ruggedness experiments to understand the functional relationship between method input variables and each of the method performance characteristics and the results are compared to the desired outcome defined in the ATP. From this, one can identify a set of operational method controls. Also, having evaluated the critical method parameters and gained a better understanding of the method through structured experimentation. [3] Addition to validating the method characteristics as per regulatory guidance, verifying the accuracy and precision provides additional understanding of the method 's measurement uncertainty and confirms conformance to the previously defined method performance requirements (ATP). This can be accomplished through a joint accuracy and precision. BF is an extremely discriminatory β1-adrenergic blocker [4]. BF is chemically: (RS)-1-[4-[[2-(1- Methylethoxy) ethoxy] methyl] phenoxy] -3-[(1 methyl ethyl) amino] propan-2-ol fumarate Figure 1. It is official in, USP. BF has similar structure to metoprolol, bopindolol, hydrochlorothiazide, atenolol [5]. Structure of BF, there is two substituents at para position of benzene provide the activity of β-selectivity, In which it has two substituents in para position of benzene which might be the activity of β- selectivity [5]. White crystalline powder of BF was soluble in water, methanol, ethanol, and chloroform. [5]. BF blocks catecholamine stimulus of β1-adrenergic receptors in the heart (cardio-selective) and Structure of BF, there is two substituents at para

position of benzene provide the activity of β-selectivity, In which it has two substituents in para position of benzene which might be the activity of β- selectivity [5]. White crystalline powder of BF was soluble in water, methanol, ethanol, and chloroform. [6]. BF blocks catecholamine stimulus of β1-adrenergic receptors in the heart (cardio-selective) and vascular smooth muscle, with decreasing the heart rate, cardiac output, systolic and diastolic blood pressure, and may be response orthostatic hypotension [6]. β-Blocker with calcium channel blocker mixture has efficacy in definite cardiovascular diseases like angina pectoris, myocardial infarction and hypertension [6]. For the decrease of workload on the heart and hence oxygen demands, so that the drug is pointed toward for secondary prevention of myocardial infarction, parallel therapy in patients with stable chronic heart failure, and for the treatment of hypertension and angina pectoris [7]. About 80% bioavailability given by BF after 10 mg oral dose [6]. The first pass metabolism of BF is about 20% and binding to serum proteins is approximately 30% [7]. The concentrations of plasma were taken in between 5 mg to 20 mg. It is contraindicated in person suffering from Psoriasis, Myasthenia Gravis, Sinus bradycardia, diabetes, depression and during Pregnancy. BF is available in combination with other drugs like HCT, AMD B, IRBE, CELI, METO T Bisoprolol is a highly selective b1-receptor antagonist devoid of any partial agonist effect (intrinsic sympathomimetic activity), vasodilatory effect, or membrane stabilising properties. It is well absorbed from the gastrointestinal tract and undergoes minimal first-pass metabolism to achieve oral bioavailability of ca. 90%. Due to its relatively long elimination half-life (10–11 h) it is suitable for a once daily administration [9]. It is cleared in equal parts unchanged by the kidney and by biotransformation in the liver [10]. Bisoprolol is marketed worldwide and its indications include hypertension, coronary heart disease, and stable chronic heart failure [10]. It is a b-blocker shown to improve survival in an outcome trial [10]. In hypertension or angina pectoris the usual therapeutic dose is 5–10 mg, and the maximum recommended dose is 20 mg. In heart failure the initial dose of bisoprolol fumarate is 1.25 mg, and it is gradually increased to 10 mg [10]. Beta-blockers are the most commonly used medicines in the therapy of hypertension; they may be used as monotherapy or concomitantly with other drugs. Despite the continuous rise in their prescription rate, bisoprolol and other b-blockers remain underused and under dosed in the treatment of heart failure and other cardiac diseases [9, 10]. It may be partially due to the limited number of available generic formulations.

The present work aims at systematic development of a simple, rapid and highly sensitive method for the analysis of bisoprolol Fumarate by QbD approach.

MATERIALS AND METHOD

Table No.1 Drug Name

Drug Name	**Bisoprolol fumarate**
Source Name	**Unichem lab. Goa**

Chemicals:

Table No.2 Chemical and Reagent

Sr .No	Reagent	Mark
1	Water	Analytical Grade
2	Chloroform	Analytical Grade
3	Ethanol	Analytical Grade
4	Methanol	Analytical Grade

Instruments: -

Table No 3. List of instruments

Sr. No.	Equipment Name	Source
1	UV visible spectroscopy	Shimadzu, Model: UV-1800
2	Digital weight balance	Shimadzu BL- 220 H
3	Hot air oven	Nisco company
4	Sonicator	The Ultrasonics PCi Analytics sonicator

DRUG AUTHENTIFICATION

Melting point (M.P)

Sample obtained was characterized for melting point of the substance. The melting point was determined by introducing small amount of substance in capillary and constant heat was applied. The drug substance was tested in the temperature range of 100~102ºC and the melting point were noted.

Solubility

The solubility of drug sample was tested in various solvents like Soluble in methanol, water (0.1 mg/ml), ethanol (8 mg/ml), ethanol, chloroform, the observed results were then compared with drug profile.

B. **Solution Preparation Method:**

Preparation of Stock solution:

Preparation of standard stock solution of Bisoprolol fumarate:

A stock standard solution was prepared by dissolving 0.010 mg of bisoprolol fumarate in a 10 ml of distilled water to obtain a concentration of 1000 µg/ml. appropriate concentration of 10 µ g/ml was prepared and scanned in the UV-visible over the range 400– 200 nm; Standard calibration solutions were prepared by dilution of the stock solutions using the diluent. These solutions were considered at six different levels which were 2 µg/ml,4 µg/ml,6 µg/ml,8 µg/ml,10 µg/ml,12 µg/ml were prepared in diluent the calibration curves for melatonin was constructed by plotting the peak area against the drug concentration.

Selection of detection wavelength:

Fixing of wave length

After selecting the suitable solvent, the fixing of the λ max for the proposed method is very important. This can be done by scanning the drug sample (Bisoprolol fumarate) solution in distilled Water in the range of 400nm-200nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λ max for the drug. And in the proposed method for Bisoprolol fumarate drug shows maximum 223 nm. With more linearity, repeatability (ruggedness) and the λmax was fixed as 223nm

Linearity and range:

For linearity study from the working standard at different concentration 2, 4, 6, 8, 10 and 12 µg/ml of drug solution were placed in 6 different 10ml volumetric flask volume was made up to the mark with bisorolol fumarate . Absorbance was measured at 223nm. The obtained data of absorbance of standard stock solution presented in Table No 10-calibration plot represented by Figure

Accuracy and recovery study:

This study was carried out using the stock solution (100µg/ml). Take three concentrations 8 µg/ml, 10µg/ml, and 12µg/ml. And take six reading of these concentrations. Calculate the % Relative Standard Deviation (RSD) of the concentration. Results within the range of ensuring an accurate method as well as indicate non-interference with the excipients of formulation. The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of Bisoprolol Fumarate solution of the drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods

Intra-day precision (repeatability) and inter-day precision study (intermediate precision):

The standard stock solution of Bisoprolol was Prepared. Prepare the three concentration of (8, 10, and 12 µg/ml), by using mobile phase methanol. Take λ max at the intraday and inter day. Calculate the % RSD. Variation of results within the day (intra-day), Variation of result between days (inter day) were analyzed. Intraday precision was analyzing Bisoprolol Fumarate for three times in the same day at 223nm. Inter-day precision was determined by analyzing the drug different day for three days at 223nm. Precision data for Bisoprolol Fumarate at 223nm.

Reproducibility:

Reproducibility is assessed by mean of an inter laboratory trial. The absorbance readings were measured at 223nm at different laboratory using another spectrophotometer and the value obtained were evaluated using t-test to verify their reproducibility data for Serotonin at 223nm is recorded.

Limit of Detection & Limit of Quantitation:

The limit of detection and quantification of drug are calculated with the standard deviation and slop.

$$LOD = \frac{3.3 \times \sigma}{S} \quad \& \quad LOQ = \frac{10 \times \sigma}{S}$$

Where,

σ = standard deviation

S = slope of calibration curve

Stability of Sample:

Samples prepared for repeatability study were preserved for 24hours at room temperature 28°C and

analyzed on the following day to test for short-term stability. The Bisoprolol fumarate sample of 4µg/ml drug solution was prepared by suitable dilution with diluents and absorbance were taken at 223nm against the blank. The stability of sample was found to be more than 10 hrs.

Acid degradation:

The preparation of 0.01N hydrochloric acid (HCl) was done by diluting 0.085 ml of conc. HCI to 100 ml of distilled water. was accurately weighted and was transferred to a labeled round bottomed flask. Reflux the sample for 2 hrs. And pipette out 1ml to 10 ml volumetric flask and adjust by distilled water by water, detect at 223 nm on uv visible spectroscopy

Base degradation:

The 0.01N Sodium Hydroxide (Noah) was prepared by dissolving 0.04 gm of sodium hydroxide pellets in 100 ml of distilled water. The solution was standardized with 0.01 N HCl as per Indian Pharmacopoeia (I.P). Bisoprolol fumarate was accurately weighted and was transferred to a labeled round bottomed flask. Reflux the sample for 2 hrs. And pipette out 1ml to 10 ml volumetric flask and adjust with by distilled water. by water. Detect at 223 nm on uv visible spectroscopy

Neutral condition:

Weight accurately 10 mg drug and transferred in to100 ml water in round bottom flask. Reflux it for 2 hours. Pipette out 1ml into 10 ml volumetric flask and adjust with mobile phase. Detect at 223 nm by water. Detect at 223 nm on uv visible spetrocopy.

Photo stability study: Photo stability was performed by placing 10 mg of Serotonin in daylight for 24 hours. The samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml sample diluted up to 10 ml by water.detect at 223 nm on uv visible spectroscopy

Dry heat:

Standard bisoprolol fumarate was placed in an oven at 60°C for 2 hours to study dry heat degradation. 10 mg drug samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml and were diluted up to 10 ml by water.

Assay Procedure -

Take weight of 10 tablets of any brand of bisoprolol Fumarate tablet. Crush the tablet in the motor pestle. Accurately weigh the quantity of powder equivalent to 10mg of drug in 100ml volumetric flask and add bisoprolol fumarate to adjust the volume up to 100ml. Pipette out the 1ml into 10 ml volumetric flask make the volume with water to get conc 10µg/ml and analyze the reading on UV visible spectroscopy. Calculate the percentage purity of tablet.

RESULT AND DISCUSSION

Preliminary solubility study of drug:

Solubility of the drug was determined at 28°C. A small quantity of standard drug was dissolved in different solvents. Implementation of QbD approach for the Spectrophotometric method development as per ICH Q8(R2) guidelines for estimation of bisoprolol fumarate by varying various parameters and these variable parameters were designed as per Ishikawa. Critical parameters for the development of zero order Spectrophotometric method are considered as various solvent, sample preparation of tablet, wavelength at 221 & 223 nm, slit width as 1, scan speed and sampling interval (0.05, 0.1, 0.2, 0.5, 1.0, and 2.0)

Determination of Variable Parameters

According to QbD approach, the first step is to determine the variable parameters for the respective method. Thus, the variable parameters for both the spectrophotometric methods were designed as Ishikawa diagram (Figures 2). For all the variable parameters as stated in Ishikawa diagram, the absorbances were recorded over the concentration range according to respective method. Working solution was scanned from 400 to 200nm and three peaks were observed at wavelengths221nm, 223nm. These three wavelengths were used as variable parameters. Also, the solubility was studied in various solvents including distilled water, and methanol. The sharpness of spectra was compared for selection of critical parameter. Scan speed was varied as fast, medium, slow, and very slow over the range 400– 200nm, while slit width and sampling interval were varied in particular ranges of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0nm and 0.05,0.1,0.2,0.5,1.0 and 2.0nm, respectively. For the estimation of Bisoprolol fumarate, two types of sample preparations were selected and evaluated. Tablets were formulated as per the master formula and were used in method development. Average weight of tablets was noted and tablets were triturated. Tablet powder equivalent to average weight was taken for study and evaluated for the method development. Recovery study was carried out at three level 80% 100% and 120%.

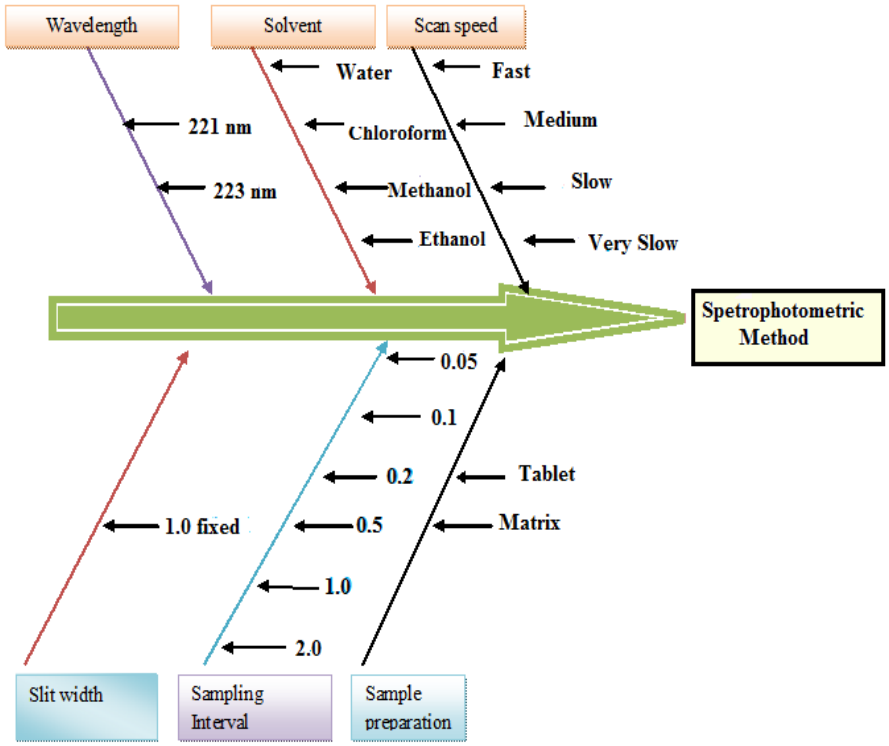

Figure No. 2 Ishikawa Diagram

Table 4: Solubility study in different Solvent by UV-absorbance

Solvent	Concentration µg /ml	Absorbance	Solvent	Concentration µg /ml	Absorbance
Water	2	1.078	Chloroform	2	0.050
	4	1.088		4	0.062
	6	1.125		6	0.078
	8	1.149		8	0.086
	10	1.175		10	0.096
	12	1.199		12	0.101
Methanol	2	0.100	Ethanol	2	0.040
	4	0.109		4	0.049
	6	0.119		6	0.054
	8	0.135		8	0.061
	10	0.155		10	0.071
	12	0.169		12	0.085

Table No.5 Effect of stirring on absorbance at λ max (223) as per stirring time

Conc. µg/ml	Effect of stirring on absorbance at λ max (223) as per stirring time.							
	0 min		2 min		4 min		6 min	
	221 nm	223nm	221 nm	223nm	221 nm	223nm	221 nm	223nm
2	0.159	0.162	0.158	0.161	0.159	0.161	0.159	0.162
4	0.183	0.187	0.184	0.187	0.183	0.186	0.183	0.187
6	0.277	0.281	0.277	0.281	0.277	0.281	0.278	0.282
8	0.362	0.364	0.360	0.362	0.360	0.361	0.360	0.362
10	0.450	0.455	0.451	0.456	0.450	0.456	0.450	0.455
12	0.535	0.542	0.536	0.543	0.535	0.542	0.536	0.543

Table No.6 Effect of stirring on absorbance at λ max (223) as per stirring time.

Solvent	Conc. µg/ml	Effect of stirring on absorbance at λ max (223) as per stirring time.							
		8 min		10 min		12 min		14 min	
		221 nm	223nm	221 nm	223nm	221 nm	223nm	221 nm	223nm
Distilled Water	2	0.159	0.162	0.0.159	0.162	0.161	0.164	0.165	0.167
	4	0.183	0.183	0.184	0.188	0.183	0.187	0.192	0.193
	6	0.279	0.283	0.279	0.288	0.285	0.288	0.281	0.285
	8	0.360	0.361	0.360	0.361	0.360	0.361	0.360	0.361
	10	0.451	0.451	0.451	0.456	0.456	0.456	0.451	0.456
	12	0.537	0.544	0.536	0.544	0.537	0.544	0.538	0.545

Table No. 11 UV absorbance as per instrumental parameter (C,Q,A) conc. 10 ppm									
Time Interval	Fast mode			Medium mode		Slow mode		Very slow mode	
	221 nm		223 nm	221 nm	223 nm	221 nm	223 nm	221 nm	223 nm
0.05	Abs.	0.337	0.360	0.357	0.377	0.359	0.381	0.369	0.390
	Time Req.	2 min	-	15 min	-	26 min	-	1 hr20 min	-
0.1	Abs	0.367	0.389	0.371	0.390	0.372	0.394	0.382	0.404
	Time Req.	1min 13 sec	-	5 min	-	13 min	-	56 min	-
0.2	Abs.	0.385	0.407	0.386	0.407	0.431	0.439	0.425	0.442
	Time Req.	40 sec	-	2 min	-	5 min	-	30 min	-
0.5	Abs.	0.385	0.408	0.389	0.406	0.431	0.435	0.425	0.441
	Time Req.	1 min	-	5 min	-	5 min	-	15 min	-
1.0	Abs.	0.384	0.395	0.355	0.370	0.389	0.401	0.399	0.405
	Time Req.	34 sec	-	46 sec	-	2 min	-	6 min	-
2.0	Abs.	0.345	0.353	0.355	0.370	0.390	0.389	0.389	0.406
	Time Req.	30 sec	-	42 esc	-	2min	-	min4	-

Table No. 7 UV absorbance as per instrumental parameter (C,Q,A) conc. 2 ppm									
Time Interval	Fast mode			Medium mode		Slow mode		Very slow mode	
	221 nm		223 nm	221 nm	223 nm	221 nm	223 nm	221 nm	223 nm
0.05	Abs.	0.145	0.146	0.146	0.146	0.146	0.147	0.147	0.147
	Time Req.	2.30 min	-	10.56 min	-	27 min	-	60.00	-
0.1	Abs	0.145	0.146	0.186	0.191	0.197	0.202	0.197	0.202
	Time Req.	2 min 10 sec	-	10 min	-	22 min	-	54 min	-
0.2	Abs.	0.196	0.202	0.197	0.202	0.191	0.198	0.190	0.198
	Time Req.	2min 10 sec	-	6 min	-	25 min	-	56 min	-
0.5	Abs.	0.194	0.200	0.198	0.204	0.197	0.203	0.198	0.204
	Time Req.	1 min 30 sec	-	3 min	-	5 min	-	15 min	-
1.0	Abs.	0.193	0.194	0.194	0.200	0.195	0.200	0.192	0.199
	Time Req.	30 sec	-	40 sec	-	1 min	-	5 min	-
2.0	Abs.	0.200	0.202	0.198	0.202	0.195	0.199	0.195	0.199
	Time Req.	10 sec	-	16 sec	-	40 sec.	-	3min 23 sec	-

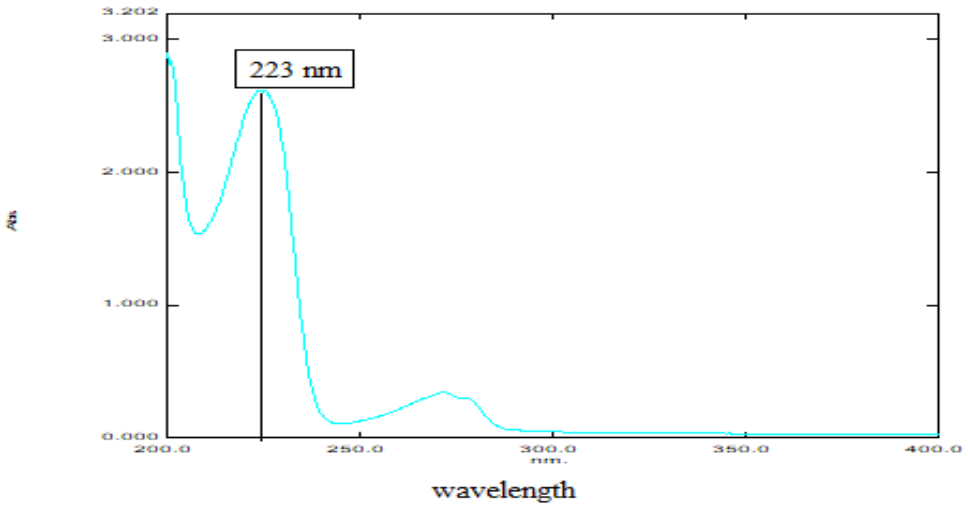

Figure No. 3: Fixing of wavelength for Bisoprolol Fumarate

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2 to 12 mg/ml was linear with a correlation coefficient (R2) 0.997.

Table No. 12 UV absorbance as per instrumental parameter (C,Q,A) conc. 12 ppm

Time Interval		Fast mode		Medium mode		Slow mode		Very slow mode	
		221 nm	223 nm	221 nm	223 nm	221 nm	223 nm	221 nm	223 nm
0.05	Abs.	0.428	0.455	0.429	0.455	0.431	0.458	0.436	0.463
	Time Req.	2.45 min	-	10. min	-	27 min	-	1hr. 20min	-
0.1	Abs	0.436	0.463	0.436	0.455	0.437	0.458	0.439	0.465
	Time Req.	2 min	-	9 min	-	26 min	-	58 min	-
0.2	Abs.	0.430	0.440	0.435	0.445	0.432	0.439	0.439	0.445
	Time Req.	1 min	-	7 min	-	8 min	-	30 min	-
0.5	Abs.	0.431	0.441	0.435	0.446	0.431	0.442	0.439	0.446
	Time Req.	35 sec	-	48 sec	-	5 min	-	15 min	-
1.0	Abs.	0.431	0.439	0.436	0.445	0.439	0.450	0.435	0.451
	Time Req.	30 sec	-	40 sec	-	3min	-	5 min	-
2.0	Abs.	0.431	0.440	0434	0.444	0.439	.0448	0.431	0.449
	Time Req.	25 sec	-	35 sec	-	1min	-	2 min	-

Table 13: Linearity and range for Bisoprolol fumarate at 223nm

Concentration (µg/ml)	Absorbance
2	0.098
4	0.245
6	0.356
8	0.459
1	0.561
1.2	0.678

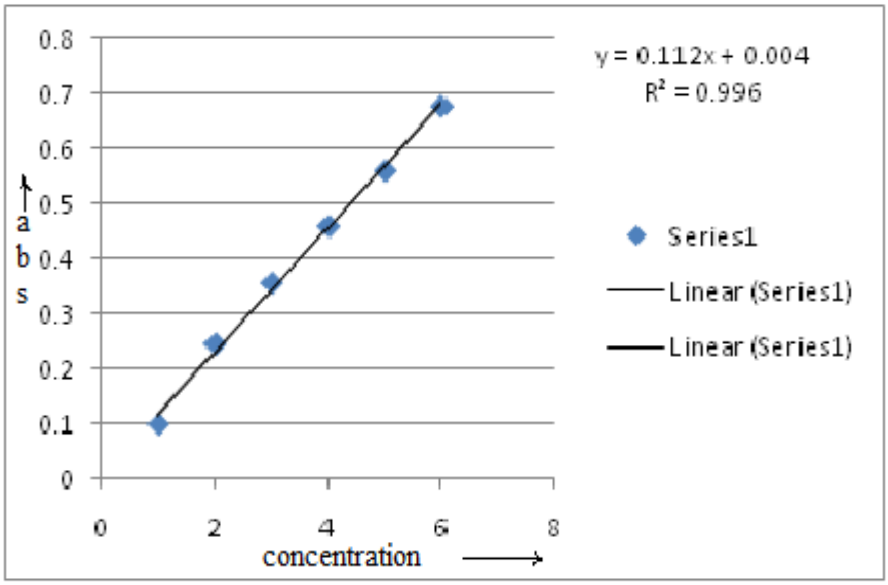

Figure No.4 Linearity of Bisoprolol Fumarate

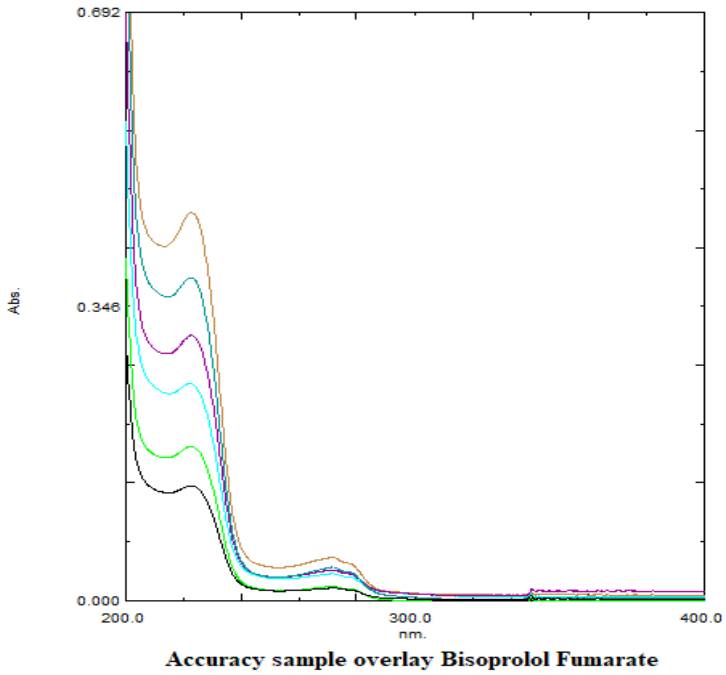

Figure No.5 Accuracy Sample Overlay of Bisoprolol Fumarate

Table No 14. Linearity Parameter

Parameter	Developed method
Conc. range	2 - 12µg/ml
Slope ± ts	0.112
*Intercept ± ts	0.004
Correlation coefficient	0.9962
LOD	0.54054
LOQ	0.1838

Intra-day precision (repeatability) and inter-day precision study (intermediate precision):

Table 15: Precision data for Bisoprolol Fumarate 223nm (Intra-Day)

Sr .no Concentration		Absorbance	
	4µg/ml	8 µg/ml	12 µg/ml
1	0.149	0.323	0.472
2	0.151	0.324	0.470
3	0.151	0.324	0.470
4	0.152	0.324	0.471
5	0.152	0.329	0.469
6	0.152	0.328	0.468
Average	0.151	0.3233	0.468
SD	0.001095	0.006055	0.005563
%RSD	0.72546	1.872	0.1886

Table No 16 -Interday precision Data

Sr .no Concentration	Absorbance		
	4 µg/ml	8 µg/ml	12 µg/ml
1	0.131	0.283	0.438
2	0.130	0.288	0.438
3	0.132	0.286	0.439
4	0.131	0.284	0.439
5	0.131	0.284	0.439
6	0.129	0.290	0.439
Average	0.1306	0.285	0.438
SD	0.001033	0.00271	0.00056
%RSD	0.790405	0.9495	0.11772

Accuracy level	% Recovery	Average	Statastical Analysis		
			mean	SD	%RSD
80%1	98.40%	99.49%	1.187	0.012	1.0118
80%2	99.49%				
80%3	100.40%				
100%1	100.06%	100.43%	1.464	0.00248	1.0674
100%2	100.01%				
100%3	100.01%				
100%4	100.02%				
100%5	100.48%				
100%6	102.48%	101.50%	1.7183	0.00702	0.408
120%1	101.18%				
120%2	101.47%				
120%3	101.77%				

Table 18 : Degradation Studies

Sr. No	Stress condition	% Degradation Observed	Remarks
1	0.1N NaOH	99.99%	Stable
2	0.1N Hcl	99.99%	Stable
3	Oven	99.99%	Stable
4	Water	99.24%	Stable
5	Photolytic	99.19%	Stable
6	Oxidation	99.05%	Stable

Implementation of QbD approach was carried out by studying variable parameters in the analytical method development. Critical parameters were extracted by observation of results as well as performing principal component analysis. Also, each method was validated according to ICH Q2 (R1) guidelines. Degradation Studies factors are mentioned in Table 18.

Table No. 19: **Critical parameters extracted for method'**

Critical parameters extracted		By Principle Component Analysis	
By observation Parameter	Extracted result	Parameter	Extracted Result
Solvent	Water	Wavelength	223 nm
		Scan speed	Fast
		Slit width	1
Sample preparation	Tablet	Sampling interval	2

Table No .20 Statistical data of Validation

Parameter	Developed method
λ max	223
Regression equation	y = 0.112x + 0.004
Conc. range	2 - 12µg/ml
Slope ± ts	0.112
*Intercept ± ts	0.004
Correlation coefficient	0.9962
LOD	0.54054
LOQ	0.1838
Intraday precision	0.72546-0.1886
Interdy precision	0.790405-0.11772

A zero-order spectrophotometric method has been developed and validated for the determination of Bisoprolol Fumarate in pharmaceutical formulation. QbD approach was carried out by varying 19 parameters and critical parameters were extracted by using principal component analysis and by observation. The extracted critical parameters are summarized in Table 19.

Melatonin followed linearity in the concentration range of 2– 12 µg/ml. The proposed method was applied for pharmaceutical formulation and percentage label claim was found to be 99.49%. The amount of drug estimated by proposed method was in good agreement with the label claim. The % recovery for bisoprolol fumarate was found to be 100.1%. The method was found to be precise as indicated by the inter-day and intra-day analysis showing % RSD less than 2. There was no any interference of excipients showing that the method was specific. Limit of detection and limit of quantitation were 0.54054 and 0.1838 µg/ml, respectively. The result did not show any statistical difference between different solvents and different wavelengths suggesting that the method developed was robust. The statistical data of validation is summarized in Table 20.

CONCLUSION

The proposed methods were found to be accurate, precise, and economical and can be useful for routine quality control analysis of Bisoprolol fumarate in pharmaceutical dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money

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