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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BISOPROLOL IN BULK MATERIAL AND IN TABLETS

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ABSTRACT

Back ground: Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Bisoprolol fumarate is the most potent beta$_1$-selective beta-blocker. It has the highest power of selective beta$_1$-activity and by blocking the beta$_1$-adrenergic receptors.

Objective: A simple, rapid, accurate and economical first order UV-derivative spectrophotometric method has been developed for estimation of bisoprolol from bulk and pharmaceutical formulation.

Method: The λ_{max} of bisoprolol in methanol was found to be 273 nm. The drug follows linearity in the concentration range 10-60 µg/ml with correlation coefficient value 0.999.

Results: The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 95% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range 98% - 102%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % RSD

value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts.

Conclusion: The above method was a rapid and cost-effective quality control tool for routine analysis of bisoprolol in bulk and in pharmaceutical dosage form.

Keywords: Bisoprolol, beta-blocker, first order derivative, UV-Spectrophotometry, methanol, validation

INTRODUCTION

Pharmaceutical analysis plays a vital role in the quality assurance and quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Analytical chemistry is the science of obtaining, processing and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much it exists **[1, 2]**. Analytical chemistry also is concerned with developing the tools used to examine chemical compositions. It is concerned with the chemical characterization of matter both qualitatively and quantitatively. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components **[3, 4]**.

Bisoprolol (chemically aryloxy propanolamine derivative) chemically, (RS)-1-[4-[[2-(1-methylethoxy)ethoxy]methyl] phenoxy]-3-[(1-methylethyl)amino]propan-2-ol **(Figure 1)**. Bisoprolol fumarate is the most potent beta$_1$-selective beta-blocker **[5, 6]**. It has the highest power of selective beta$_1$-activity and by blocking the beta$_1$-adrenergic receptors it reduces the heart rate, and contraction of the heart thus lowers the blood pressure. The molecular formula of bisoprolol fumarate is $C_{18}H_{31}NO_4$ and molecular weight is 325.443 g/mol. Bisoprolol available in fumerate salt form (Bisoprolol Fumerate – $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$, M.W. - 767 g/mol) **[7, 8]**.

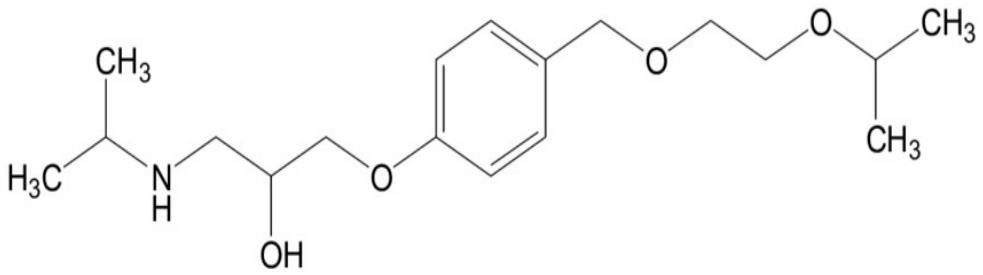

Figure 1: Chemical structure of bisoprolol

Literature survey reveals that only RP-HPLC, HPTLC and LC-MS methods were reported for the determination of bisoprolol. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. The objective of the present research work was to develop new analytical UV spectrophotometric method and its validation parameters for the proposed method according to ICH Q2 guidelines for the estimation of bisoprolol in bulk and tablet dosage form. The developed method can be applied for routine analysis of bisoprolol in bulk and pharmaceutical dosage form. On the other hand, UV spectrophotometry is fast, simple, economic and universally accepted in pharmaceutical analysis. A literature survey revealed that no UV spectrophotometric method has been reported for the estimation of bisoprolol in pharmaceutical formulations individually. Thus, the present study describes a direct, simple and accurate method for the estimation of bisoprolol in bulk and in tablet dosage forms **[9-11]**.

MATERIALS AND METHODS

Instruments used

The analysis was performed on A double beam UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. And other instruments are Schimadzu Digital Electronic Balance-BL 220H, and Ultrasonic cleaner were purchased from Life Care Equipment Pvt. Ltd., Hyderabad, Telangana, India.

Chemicals and reagents used

The pure API sample of tolvaptan was obtained as free gift sample from Hetero Labs, Hyderabad, Telangana, India. The marketed pharmaceutical dosage form of bisoprolol tablets (5 mg) was purchased from local Apollo Pharmacy, Hyderabad, Telangana, India. All reagents like HPLC grade methanol, HPLC grade water and HPLC grade acetonitrile are purchased from Hyderabad, Telangana were utilized throughout the experiment.

Selection of solvent [12]

A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile were tried based on the solubility of the drug. Bisoprolol was found to be freely soluble in methanol and double distilled water, insoluble in acetonitrile.

Preparation of standard stock solution [13, 14]

An accurately weighed quantity of bisoprolol 10 mg was transferred to 50 ml

of clean and dried volumetric flask, and dissolved in 20 ml, the final volume was made with methanol to obtain standard solution having concentration of 1000 µg/ml. 1 ml of this solution was transferred to 10 ml volumetric flask, and volume was made with methanol, it gives 100 µg/ml. These stock solutions were used to prepare further dilutions throughout the experiment.

Selection of wavelength (λ_{max}) [15]

Appropriate volume 1 ml of standard stock solution of bisoprolol was transferred into a 10 ml volumetric flask, diluted to a mark with methanol to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Analytical method validation developed [17-19]

The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH Q2 guidelines. The method was validated in order to determine the linearity, precision, accuracy, repeatability, ruggedness, LOD and LOQ of the method.

Linearity

Linearity and range different concentrations of bisoprolol solutions were prepared. The range of the solutions varies from 10% to 60% of standard concentration (µg/ml) of 1 mg. The absorbance of these solutions is noted. The absorbance of the lower-level linearity solution (10%) and the higher-level linearity solution (60%) in 6 replicates were recorded. The graph of concentration vs absorbance of linearity solutions was plotted.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Intraday precision study was carried out by preparing drug solution of six different concentrations (10, 20, 30, 40, 50, 60 µg/ml of bisoprolol) and analyzing it at five different times in a day. Interday precision study was carried out by preparing drug solution of six different concentrations (10, 20, 30, 40, 50, 60 µg/ml of bisoprolol) and analyzing it at three different days.

Repeatability

Repeatability was determined by preparing six replicates of 1 µg/ml of bisoprolol and the absorbance was measured at 273 nm.

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the %recovery was calculated.

Limit of Detection and Limit of Quantitation

The parameters LOD and LOQ were determined on the basis of response and

slope of the regression equation. The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH Q2) guidelines.

$$\mathbf{LOD = 3.3 \times \sigma/S}$$

$$\mathbf{LOQ = 10 \times \sigma/S}$$

Where,

σ = Standard deviation of the response, and

S = Slope of the calibration curve.

Ruggedness studies

Ruggedness studies were performed by preparing three replicates of 1 µg/ml of bisoprolol, analysing by two different analyst and on two different instruments and the results are reported as % RSD.

Assay for pharmaceutical formulation (Tablets)

The solution was filtered through Whatman filter paper No. 41. 0.5 ml this solution was transferred to 10 ml volumetric flask and final volume was made with methanol. It gives 0.5 µg/ml. It was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 273 nm against blank solution of methanol. Determine the amount of %bisoprolol in tablet according to the following formula:

$$\%\ \mathbf{Assay} = \frac{\mathbf{WS\ X\ AT\ X\ Sample\ D.F.\ X\ Avg.\ Wt.}}{\mathbf{AS\ X\ Standard\ D.F.\ X\ WT\ X\ LC}}\ \mathbf{X\ PS}$$

Where,

WS = weight of standard;

WT = weight of sample

AT = Absorbance of bisoprolol in the test solution,

AS = Absorbance of bisoprolol in the standard solution,

Standard D.F. = Standard dilution factor,

Sample D.F. = Sample dilution factor,

PS = Purity of working standard [%],

LC = Label claim of bisoprolol.

RESULTS AND DISCUSSION

Validated analytical methods are aimed for the estimation of bisoprolol in API and its formulation. Simple, precise, rapid, accurate methods were developed for the estimation of bisoprolol in formulation by UV-spectroscopic method.

The validated method was applied for the analysis of tablet containing 5 mg to bisoprolol drug as the label claim. The method developed was simple and rapid.

In case of UV-spectroscopic method solubility is the important parameter. Solubility parameter was studied and methanol was selected as the solvent, since it gave a maximum absorbance and a good spectral pattern when compared with other

solvents. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 273 nm. Percentage recovery, linearity, stability studies were also carried out. The above method gave a satisfactory recovery value and found to be stable, linear, hence it can be used for routine analysis of the drug formulation.

Solutions of bisoprolol and its marketed product were prepared by using methanol and UV spectrum of each was recorded by scanning between 200-400nm.

Selection of solvent

An overlain spectrum of bisoprolol and marketed product was prepared in solvent like methanol. Better absorbance was observed for both the API and formulation when methanol is used as a solvent as shown in the **Figure 2**. Hence, methanol was selected as solvent for present study.

Absorbance maxima (λ_{max})

The absorbance maximum of bisoprolol was found to be 273 nm. The UV spectrum for bisoprolol is depicted in **Figure 2**.

Method validation

The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Linearity

Standard solutions of bisoprolol in the concentration range of 10 to 60% were observed in UV-Spectroscopy.

A graph of absorbance (on Y-axis) versus concentration (on X-axis) was plotted and calibration graph was shown in **Figure 3**. The regression equation was found to be Y=0.0108x + 0.0048, Correlation coefficient was 0.999.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise for the determination of the pure form. The intra and interday precision results were mentioned in **Table 1 and 2** respectively.

Repeatability

Repeatability was determined by analyzing 10 µg/ml concentration of bisoprolol for six times with % RSD < 2 which illustrated in **Table 3**.

Accuracy

Accuracy shall be determined by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The mean recovery was found between 98 - 102% and % RSD between 0.7 - 1.0. The results are given in **Table 4**.

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

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD and LOQ values are 0.48 and 1.46 µg/ml respectively. The results are illustrated in **Table 5**.

Ruggedness studies

This study was performed by analyzing 10 µg/ml of bisoprolol by two different analysts and on two instruments, results of the study were given in **Table 6** and % RSD obtained was less than two which is within the acceptance limits.

Assay for pharmaceutical formulation

The percentage recovery for bisoprolol tablet formulation was found to be 95 - 101% enlisted in **Table 7**. The results for assay are within acceptable limit.

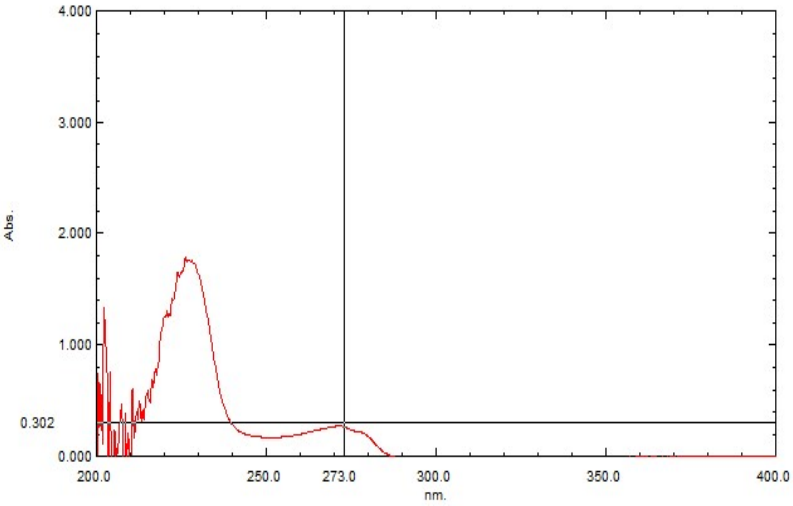

Figure 2: Absorption spectrum of bisoprolol in methanol

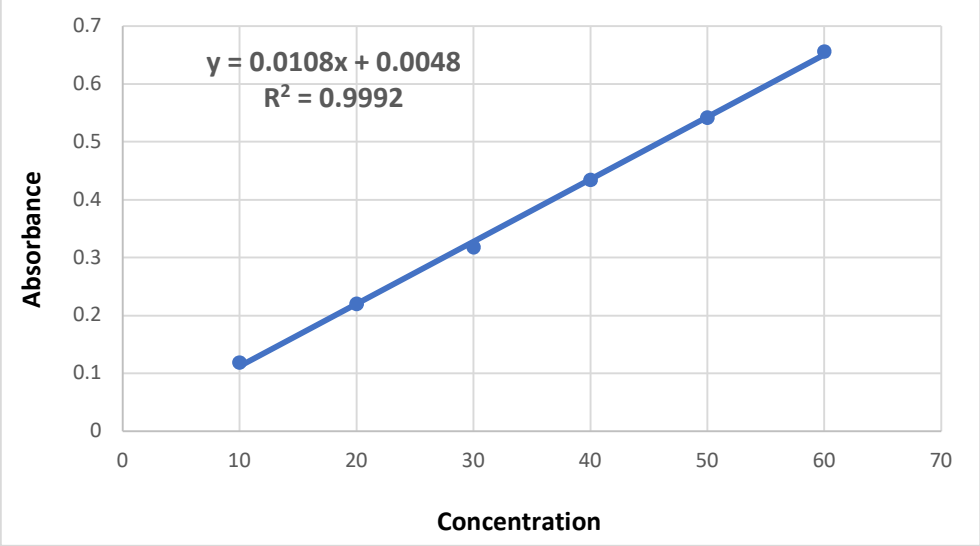

Figure 3: The graph of concentration vs absorbance of linearity solutions (bisoprolol)

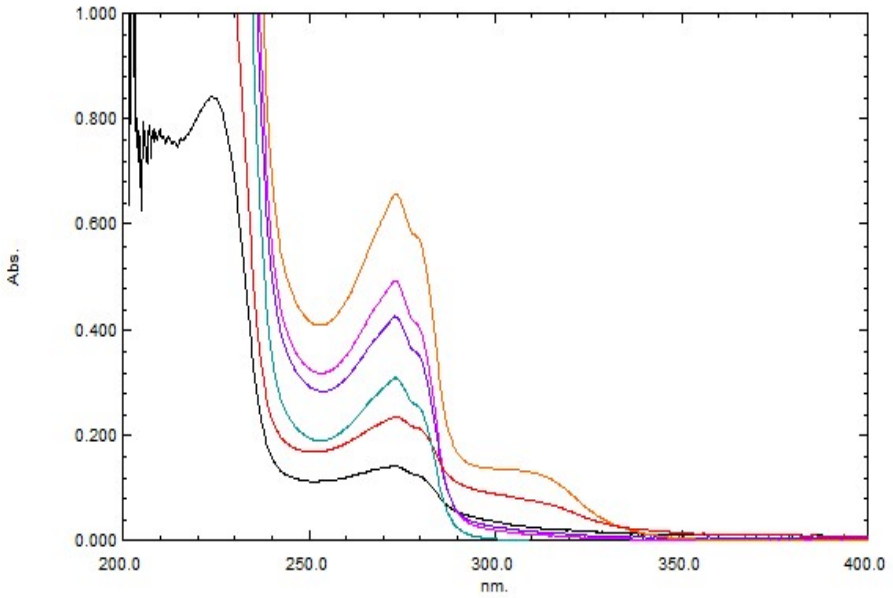

Figure 4: Overlay spectra of bisoprolol at 273 nm

Table 1: Intraday precision

Conc. in µg/ml	Interday Precision	% RSD
	Absorbance mean ± S.D. (n=3)	
10	0.104 ± 0.001581	0.015203
20	0.2264 ± 0.002074	0.009159
30	0.3616 ± 0.00305	0.008434
40	0.3994 ± 0.004219	0.010563
50	0.6276 ± 0.002966	0.004727
60	0.6268 ± 0.001924	0.003069

Table 2: Interday precision

Conc. in µg/ml	Interday Precision	% RSD
	Absorbance mean ± S.D. (n=3)	
10	0.1396 ± 0.02303	0.016491
20	0.2736 ± 0.00114	0.004167
30	0.3894 ± 0.002966	0.007618
40	0.4896 ± 0.001673	0.003418
50	0.7048 ± 0.003114	0.004419
60	0.7694 ± 0.020744	0.026961

Table 3: Repeatability studies

Conc. in µg/ml	Absorbance at 273 nm	Absorbance Mean	S.D.	% RSD
10	0.119	0.1196	0.00081	0.0067
10	0.119			
10	0.119			
10	0.120			
10	0.121			
10	0.120			

Table 4: Recovery studies

Drug	Amount taken	Amount added	Total amount found	Mean recovery	% RSD
Bisoprolol	60mg	80mg	74.3mg	100.07	0.0158
		100mg	60.32mg		
		120mg	50.32mg		

Table 5: LOD and LOQ

Drug	LOD	LOQ
Bisoprolol	0.48 µg/ml	1.46 µg/ml

Table 6: Ruggedness of bisoprolol

Parameter	Conc. (µg/ml)	Absorbance	Absorbance Mean ± S.D. (n=3)	% RSD
Different Analyst	10	0.115	0.118 ± 0.0030	0.025
		0.121		
		0.119		
Different instrument	10	0.114	0.118 ± 0.0036	0.030
		0.119		
		0.121		

Table 7: Assay of bisoprolol tablets

Drug	Labelled Amount	Amount found	% Label claim	% RSD
Bisoprolol	5mg	4.75mg	95%	0.0375

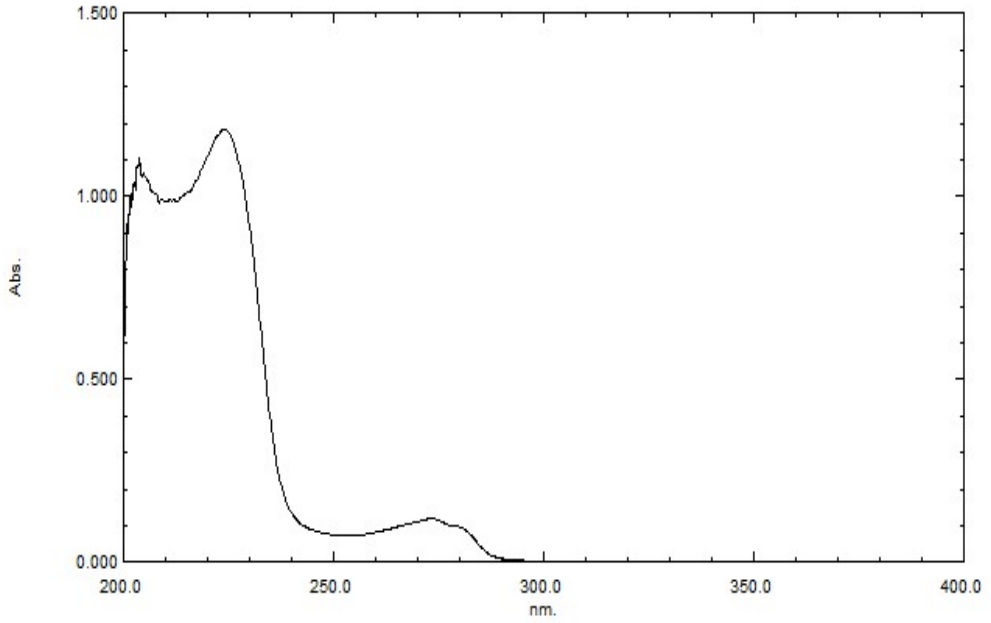

Figure 5: Formulation spectra of bisoprolol at 273nm

Table 8: Summary of validated parameters

Parameter	Method
λ_{max}	273 nm
Beers law limit	10-60 µg/ml
Correlation coefficient (r^2)	0.999
Molar absorptivity	$3.41x10^4$ L mol^{-1} cm^{-2}
Regression equation (y=mx+c)	Y=0.0108x + 0.0048
Slope (m)	0.01076
Intercept (c)	0.0048
Accuracy	98 – 102%
Precision	< 2
LOD	0.48 µg/ml
LOQ	1.46 µg/ml

CONCLUSION

From present research work, it is concluded that the first order derivative and is economical and reproducible. The method was developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of bisoprolol from pharmaceutical dosage form (Tablets). It is inferred that the methods were found to be simple, accurate, precise and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The results showed that the recovery of marketed product by the proposed method was satisfactory. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound. The result obtained from the validation parameters met the ICH Q2 and USP requirement as well as obeys Beer's law.

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Declarations

Author contributions

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

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