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VALIDATED AND STABILITY INDICATING LIQUID CHROMATOGRAPHY METHOD FOR QUANTIFICATION OF BISOPROLOL FUMARATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple and accurate liquid chromatographic method was developed and validated for the analysis of bisoprolol fumarate in tablets. Chromatographic separation was achieved on a C18 column utilizing a mobile phase of buffer/acetonitrile (75:25, v/v, pH 5.6) at a flow rate of 1.0 mL/min. The separation was performed at room temperature. Detection was carried out at 226 nm, using a diode array detector. The developed method was statistically validated for the linearity, accuracy, limit of detection, limit of quantitation, precise and specificity. The specificity of the method was ascertained by forced degradation studies; the degraded products were well separated from the analyte. The mean recovery for bisoprolol fumarate from tablets ranged between 99.87-100.43%. The proposed method is also found to be precise and robust. The method can be used for routine quality control analysis.

Keywords: Bisoprolol Fumarate, Tablets, HPLC and Stability Indicating

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INTRODUCTION

Bisoprolol is a cardio selective beta-blocker. It is given as the fumarate in the management of hypertensive and angina pectoris. Chemically it is (±)-1-[4-[[2-(1-methyl ethoxy) ethoxy] methyl] phenoxyl-3-[(1-methyl ethyl) amino]-2-propanol (*E*)-2-utenedloate (2:1) (salt) [1, 2]. A literature survey revealed that few analytical methods are available for the estimations of Bisoprolol Fumarate from dosage forms[3-5] and from human plasma[6-8]. The reported methods include HPLC[5], the spectrophotometric[4] and HPTLC[3] method of analysis. There is also liquid chromatographic-tandem mass spectrophotometric[6, 7] and LC-ESI-MS[8] methods reported for the determination of Bisoprolol in human plasma. The earlier reported methods were either less sensitive or involved costlier techniques. The International

Conference on Harmonization (ICH) guidelines require that stress testing be carried out to elucidate the inherent stability characteristics of the active substances. It suggests that degradation products that are formed under a variety of conditions should be identified and the degradation pathway established. It is states that testing should include the effect of temperature, humidity, oxidation, photolysis, and acid and base hydrolytic conditions[9]. An ideal stability-indicating method is the one that quantifies the drug *per se* and also resolves its degradation products. Stability is considered to be one of the most important criteria in pharmaceutical quality control. Only a stable preparation would promise precise delivery of the drug to the patients. Expiration dating on any drug product is based on scientific studies at normal and stressed conditions[10]. With this background, an attempt has been made to develop

and validate a stability-indicating RP-HPLC method, for the accurate quantitation of Bisoprolol fumarate in bulk drugs, in the presence of its degradation products.

MATERIALS AND METHODS

Materials: Pure bisoprolol fumarate (BF) was obtained as gift sample from Genovo Development Services Ltd., Bangalore, India. Acetonitrile was of HPLC grade and purchased from Spectrochem, Mumbai. Ammonium dihydrogen orthophosphate was of AR grade and purchased from Merck, India, and other chemicals were of analytical grade from SD Fine Chemicals, India. Deionized and ultra pure water used in all experiments was obtained from Milli-Q System (USA). The 0.45μm Nylon pump filter was obtained from Advanced Microdevices (Mumbai, India). Formulations of BF used for the study were tablets (BISOCAR, Rusan H. Care, India) containing 2.5 mg and 5.0 mg of BF and were procured from local market.

Equipment: The analysis was performed on HPLC equipment (Shimadzu-1200 series, Japan) consisted of a LC-20AD solvent delivery system, a diode array detector (SPD-M20A) and G1313A auto sampler. Chromatograms were analysed using Empower^{TM}2 chromatography data software provided with the system.

Chromatographic condition: The chromatographic separation was achieved on a reverse phase Protosil, Chromo band C_{18} (250 x 4.6 mm, 5 μm particle size) column. Mobile phase consisted of a mixture of buffer and acetonitrile at a ratio of 75:25 (Buffer; pH 5.6, adjusted with orthophosphoric acid). The flow rate was set at 1.0 mL/min. The injection volume was 10 μL and the wavelength was set to 226 nm.

Preparation of Mobile phase: Accurately weighed about 6.6 g of ammonium dihydrogen orthophosphate, transferred to 980 mL of water, add 2 mL of triethyl amine and adjusted the pH to 5.6 with orthophosphoric acid, and made up to 1000 mL with water. Before use, the mobile phase was degassed by an ultrasonic bath and filtered through a 0.45 Nylon filter.

Preparation of the standard solutions: Standard solution (1000 μg/mL) was prepared by transferring 100 mg of working standard into a 100 mL volumetric flask, 50 mL water was added, and the mixture was sonicated to dissolve and make up the volume with water. Aliquots of these standard solution was transferred using A-grade bulb pipette

into 100 mL volumetric flasks and made up to volume with mobile phase to get final concentrations of 25 - 100 μg/mL. The solutions were then filtered through a 0.45 Nylon filter. The filtered solutions were then injected into HPLC system.

Method Validation: The method was validated for linearity, limit of detection (LOD), and quantification (LOQ), system suitability, precision, accuracy, specificity, and robustness in accordance with the ICH guidelines.

Linearity: By appropriate dilutions of the BF standard solution with the mobile phase, six working solutions ranging between 25 and 100 μg/mL were prepared. Each solution was injected in triplicate and linearity was evaluated by linear-regression analysis.

Limit of detection and quantification: Limit of detection (LOD) and limit of quantification (LOD) were calculated based on the ICH guidelines.

Accuracy: Accuracy was assessed by recovery studies of the method at three different concentrations (corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. The results were expressed as mean percentage recovery and relative standard deviation (%RSD).

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of BF at a concentration of 25, 50 and 75μg/mL. Determinations were performed with three replicates on the same day as well as on three consequent days.

Robustness: The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions. Robustness of the developed was investigated by varying the chromatographic conditions such as change of organic strength (±2%), flow rate (±10%), and pH of the buffer (±0.1 units).

Solution stability: Stability in solution was evaluated for the standard solution and the test preparation. The solutions were stored at 5º and at ambient temperature without protection of light and tested after 12, 24, 36 and 48 h. The responses for the aged solution were evaluated by comparison with freshly prepared solutions.

System suitability: The suitability of the chromatographic system was tested before each stage

of validation. Five replicate injections of standard preparation were injected and asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Stability studies of Bisoprolol fumarate:

In order to determine whether the analytical method and assay were stability-indicating, pure drug was stressed under various conditions to conduct forced degradation studies.

Acid- and Base- induced degradation: Acid-induced degradation was performed by adding 5 mL of stock solution (1000µg/mL) of bisoprolol fumarate to 5 mL 0.1N hydrochloric acid and was refluxed for 1 h at 60°C. The resultant solution was diluted to obtain 50µg/mL and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample. Base-induced degradation was performed by adding 5 mL of stock solution (1000µg/mL) of bisoprolol fumarate to 5 ml 0.1N sodium hydroxide and was refluxed for 1 h at 60°C. The resultant solution was diluted to obtain 50µg/mL and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation: To study the effect of the oxidizing conditions, 5 ml of stock solution (1000µg/mL) of bisoprolol fumarate was added 5 mL of 1% hydrogen peroxide solution and solution was refluxed for 1 hr at 60°C. For HPLC study, the resultant solution was diluted to obtain 50µg/mL and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation: Stress testing under neutral conditions was studied by refluxing the drug in water for 1 h at 70°C. For HPLC study, the resultant solution was diluted to get 50 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolysis: The photochemical stability of the drug was also studied by exposing the stock solution (1000 µg /mL) to UV-Light for 200 Watts/m^{2}. The resultant solution was diluted to obtain 50 µg/mL of BF and 10 µL of the solution was injected into the HPLC, and chromatogram was recorded.

Thermal Degradation: The standard drug was placed in oven at 80º for 3 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50 µg/ml solution and 10 µl were injected into the

system and the chromatograms were recorded to assess the stability of the sample**.**

Assay procedure for Tablets: To determine the content of BF in Tablets (BISOCAR, Rusan H. Care; labeled claim: 2.5 & 5 mg/tablet), twenty tablets were weighed and the content was finely powdered. The powder equivalent labeled claim of BF, into a 250 mL volumetric flask, add about 170 mL of diluents, and sonicated for 25 min with occasional shaking, make up to the mark with mobile phase, and mix well. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant solution. The experiment was performed six times under the chromatographic conditions   described above. The peak areas were measured at 226 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

RESULTS AND DISCUSSIONS

The chromatographic conditions were optimized to develop a stability indicating assay method for bisoprolol fumarate in tablet dosage forms. The basic chromatographic conditions were designed to be simple and easy to use and reproduce and were selected after testing the different conditions that affect HPLC analysis, for example column, aqueous and organic components of the mobile phase, proportion of mobile phase components, detection wavelength, diluents and concentration of analyte. The Protosil, Chromo band C$_{18}$ column was used because of its advantages of high resolving capacity, better reproducibility, low-back pressure, and low tailing. The proportion of the mobile phase components was optimized to reduce retention time and enable good resolution of bisoprolol fumarate from the degradation products. A detection wavelength of 226 nm was selected. Detection at 226 nm resulted in good response and good linearity.

The calibration curve was prepared by plotting the peak area of BF against drug concentration (µg/mL) and was linear in the range of 25-100 µg/mL. The data were subjected to least-square linear regression analysis to calculate the calibration equation and correlation coefficient. The regression equation was found as $Y=276762X+164954$ (r = 0.9998). The results show that there is an excellent correlation between the peak area and the concentration of BF in the range tested. The limit of detection, with a signal to noise ratio of 3:1, was found to be 0.02µg/mL. The limit of quantitation, with a signal to noise ratio of 10:1, was found to be 0.05µg/mL. Results from the

linear regression analysis with system suitability data were listed in Table 1.

The results of intra-day and inter-day precision studies were shown in Table 2. They revealed that % RSD values for intra-day studies ranged between 0.43-0.68% and for inter-day precision between 0.44-0.82%, which are within the permissible limits of 2.0%. To examine the accuracy of the method, recovery studies were carried out by standard addition method. The results were shown in Table 2. The average percent recoveries obtained as 99.87-100.43%, indicating that the method was accurate.

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value. System suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions. During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 48 h. Before each measurement of validation data a system suitability test was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates and RSD (%) of peak area observed for a standard solution. The values obtained were satisfactory and in accordance with in-house limits.

The specificity of the method was also evaluated by checking the peak purity of the analyte peak during the forced degradation study. The peak purity of the bisoprolol fumarate peak under different stress conditions was 1.00, which is satisfactory and indicates there was no interference with the analyte peak from degradation products. Major degradation up to 23.25% occurred under oxidizing conditions. Under alkaline conditions the drug was degraded by approximately 12.74%. The drug was approximately 15.62% degraded under acidic conditions. The drug was degraded 10.65% under thermal condition and 14.39% degradation occurred under photolytic conditions (Figure 2).

The proposed method was applied to the analysis of marketed product and the results obtained were given in Table 3. The blank solution was prepared containing the components indicated in tablets except active principle. No interference was observed from the tablet excipients. The results were indicated that the method is suitable for routine analysis of bisoprolol fumarate in pharmaceutical dosage forms.

CONCLUSION

The developed HPLC method is accurate, precise, reproducible, robust, and stability indicating. The method is linear over a wide range, economical and utilizes a mobile phase, which can be easily prepared. All these factors make this method suitable for estimation of BF in pharmaceutical dosage forms. The method can also be used for the routine analysis of BF in bulk drug and pharmaceutical formulations without interference.

Table1. Results from regression analysis and system suitability of Bisoprolol fumarate

Description	Value
Retention time (min)	9.5
Linear range (µg/mL)	25-100
Limit of detection (µg/mL)	0.02
Limit of quantification (µg/mL)	0.05
Regression line	Y=276762X+164954
Correlation coefficient (r)	0.9998
Theoretical plates	13524
Tailing factor	1.13

Table2. Results of recovery studies and precision

Actual Conc. (µg/mL)	% Recovery		Precision			
			Intra-day		Inter-day	
	Mean±SD	%RSD	*Mean±SD	%RSD	*Mean±SD	%RSD
25	99.87±0.8354	0.84	25.08±0.2043	0.68	25.01±0.2043	0.82
50	100.11±0.978	0.98	49.91±0.2829	0.57	49.96±0.2802	0.56
75	100.43±0.9696	0.96	75.09±0.3258	0.43	75.11±0.3279	0.44

*Concentration (µg/mL)

Table 3: Analysis of bisoprolol fumarate in tablets

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
BISOCAR	2.5	100.11±0.6866	0.6859	0.2803
	5.0	100.41±0.8671	0.8636	0.354
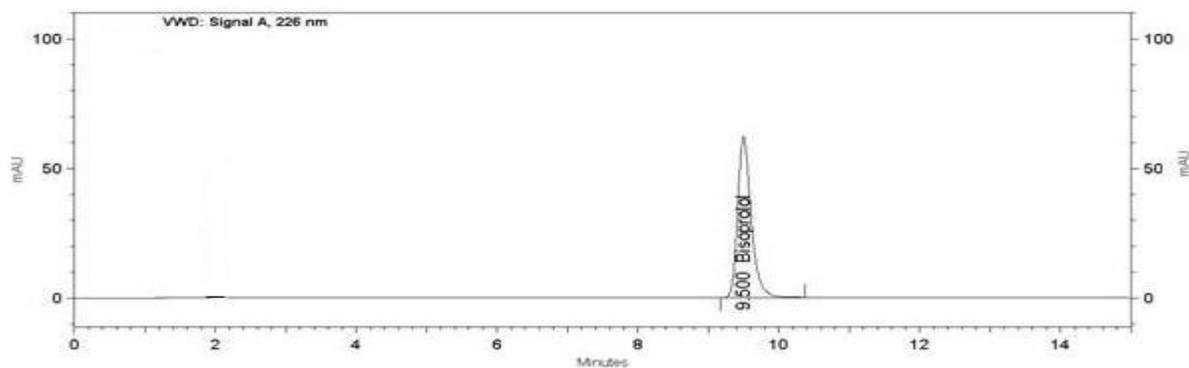
Figure 1: A typical chromatograph of bisoprolol fumarate
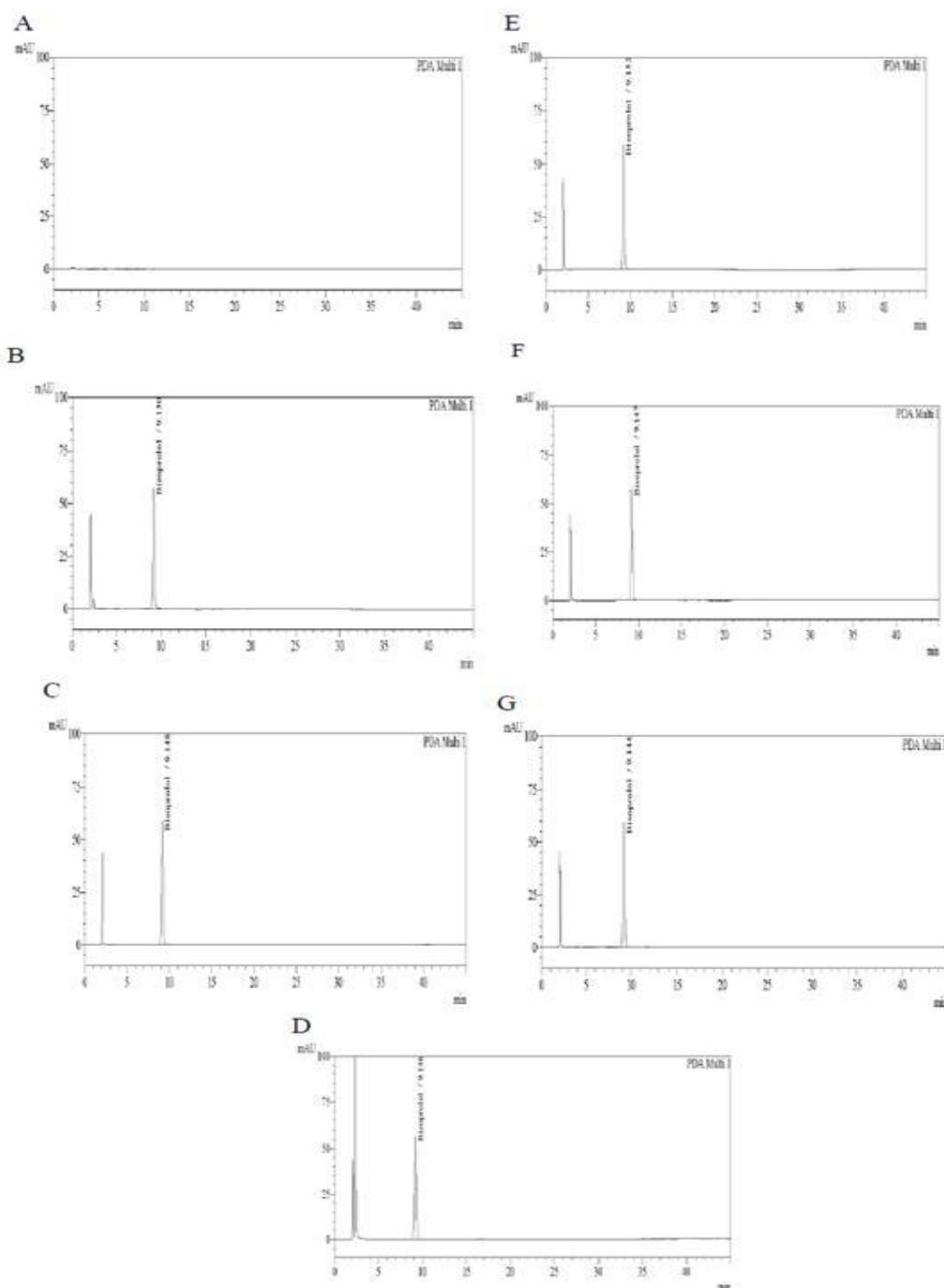
Figure 2: Chromatograms corresponding to BF solution subjected to A) Blank, B) Acid hydrolysis, C) Base hydrolysis, D) Oxidation, E) Neutral, F) Thermal, G) Photolysis

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