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Analytical High Performance Liquid Chromatography Method for Estimation of the Bisoprolol Fumarate

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KEYWORDS
Quality by design, Bisoprolol Fumarate, HPLC.

ABSTRACT:

Introduction: Quality by Design (QbD) guidelines from ICH address pharmaceutical drug ingredient and product research, method development, and validation. Analytical techniques aid QbD application in pharmaceutical development, manufacturing, and quality evaluation. Risk assessment, process monitoring, and analytical testing are crucial steps.Bisoprolol fumarate is a white, crystalline powder used in treating coronary heart disease, hypertension, and chronic stable heart failure. Its molecular formula is C18H31NO4, and its effectiveness in treating myocardial infarction with cardiac insufficiency is being investigated for its impact on cardiac function.

Objectives: This study aimed to enhance the stability indicating chromatographic method and validation parameters for determining Bisoprolol fumarate in the presence of degradation products and impurities, ensuring purity and stability of the bulk drug using HPLC, in compliance with ICH recommendations.

Methods: The central composite design is a popular factorial design in the Response Surface Model (RSM), used to estimate the accurate curvature of the Response Surface. It involves a point at the center and a star point outside the domain.The study examined the impact of mobile phase composition, flow rate, retention time, peak area, theoretical plate number, and peak symmetry on the performance of an analytical method.The mobile phase was created by mixing methanol with water containing orthophosphoric acid, filtering, and sonicating for 10 minutes to ensure thorough mixing and eliminate trapped air bubbles.A 1000 ug/ml standard solution was prepared by dissolving bisoprolol fumarate in methanol, and samples were prepared by dilution to create varying concentrations for analysis.

Results: The HPLC method was validated according to ICH guidelines and was found to be linear, accurate, and precise for Bisoprolol fumarate. The % recovery of Bisoprolol Fumarate was found to be 108.08 %

Conclusions: This study introduces a new, rapid, and sensitive HPLC method for determining bisoprolol fumarate in its pure and dosage forms. It simplifies the extraction process and utilizes cost-effective equipment, solvents, and reagents. This method offers a faster, more efficient, and cost-effective analytical tool for research and pharmaceutical settings.

1. Introduction

Quality by Design (QbD) guidelines Q8 Q9 and Q2 of ICH address QbD, which is well recognized in the field of pharmaceutical drug ingredient and product research, method development and validation. The application of Quality by Design (QbD) in process pharmaceutical development, manufacturing and development is greatly aided by analytical techniques. In the creation of pharmaceuticals, risk assessment, process monitoring and control, ongoing quality evaluation of finished products, and analytical testing are also crucial steps. As a quality indicator in the chromatographic technique, the development of experiment design to method development paradigm of Quality by design.[1]

ICH Q2, Q8, and Q9 refer to key guidelines in pharmaceutical development and quality control.

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ICH Q2 : Analytical Procedures Validation - It provides instruction for validating analytical processes with a focus on robustness, specificity, accuracy and precision.

ICH Q8 : Pharmaceutical Development - Guides the development of a pharmaceutical product by emphasizing the importance of a quality-by-design approach.

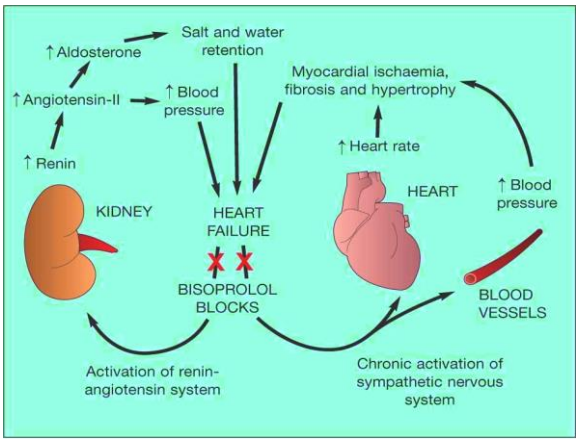
ICH Q9: Quality risk management - Focuses on the systematic process of quality risk management to enhance the quality of pharmaceutical products.[2]

The powder of Bisoprolol fumarate is white and crystalline, with high solubility in methanol and water, free solubility in ethanol, glacial acetic acid, and chloroform, and only minimal solubility in ethyl acetate and acetone.

The (RS)-1-{4-[(2-Isopropoxyethoxy)methyl]phenoxy}-3-(isopropylamino)propan-2-ol is bisoprolol and the molecular formula is C18H31NO4. The molecular weight of bisoprolol fumarate is 766.96.

Treatments for coronary heart disease, hypertension, and moderate-to-severe chronic stable heart failure sometimes involve the use of bisoprolol. Analyzing the effectiveness of bisoprolol in treating myocardial infarction with cardiac insufficiency and its impact on cardiac function is the goal of this investigation. The synthetic beta-1 selective (cardioselective) adrenoceptor blocker is called bisoprolol fumarate.[3]

The way Bisoprolol affects the heart and kidney is used



in treating hypertension and its related conditions. The bisoprolol fumarate blocks the B1 receptor and gives a negative inotropic and chronotropic effect which decreases blood pressure, heart rate and cardiac contraction on renal juxtaglomerular cells it acts by decreasing renin release and prevents the renin–angiotensin system from activating.

Treatment of High Blood pressure (hypertension) involves the combination of bisoprolol fumarate and hydrochlorothiazide. The heart and arteries work harder when blood pressure is high Bisoprolol fumarate and Telmisartan treatment improves blood pressure control in patients with hypertension after failure of monotherapy.[4]

2. Objectives

The goal of this work was to increase stability indicating chromatographic approach for the determination of Bisoprolol fumarate in presence of degradation products and associated impurity for the evaluation of purity of bulk drug and stability of its bulk dosage form the usage of HPLC. The technique was validated in compliance with ICH recommendations and its up-to-date international convention.

The approach needs to be simple, correct, precise repeatable and stability indicating; also, it ought to decrease the length of evaluation and have to be appropriate for routine determination of Bisoprolol fumarate in the Tablet dosage form.

3. Methods

Material

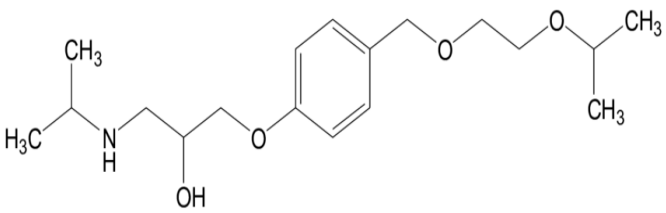
Chemical:

Bisoprolol Fumarate, Methanol, Distilled Water , 0.1% Orthophosphoric acid as buffer.

Apparatus:

The instrument used for this experimentation was Agilent Model 1100 series High performance liquid chromatography the chromatography column used was Zobrax SB C18 (0.5um, 4.6 * 250 mm).

Structure



Bisoprolol fumarate

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Method

Central Composite Design

The central composite design is a widely used factorial design in the Response Surface Model (RSM). It includes factorial, full, or fractional designs and is well-known for its popularity and extensive application. The point at the center of the experimental domain & the star point outside the domain are used to make it possible to estimate the accurate curvature of the Response Surface The center point is arranged with the axial point called as star point .It is always been difficult to select an accurate experimental design for such kind of interpretation the CCD is an excellent choice with accuracy.

This study investigated the influence of various factors on the performance of our analytical method for measuring a specific compound. We focused on independent variables: mobile phase composition (methanol/water ratio), and flow rate. Dependent variables included retention time, peak area, number of theoretical plates and peak symmetry.

We selected a suitable column based on our chosen analytical method. We then prepared and tested eight different mobile phase compositions with various methanol/water ratios and flow rates. These experiments, based on a central composite design, provided the data for subsequent validation of the optimized method.[5]

Table 1: Factor considered for the study by software used

Run	Standard	Factor 1 Methanol	Factor 2 Water	Flow rate ml/min	RT Retention Time	PA Peak Area	TP Theoretical Plate	TF Tailing Factor
1	4	52	48	0.9	3.194	1159.02100	5850	0.74
2	6	52	48	0.8	3.604	1436.91125	6712	0.71
3	1	50	50	0.7	4.191	1611.35901	6610	0.71
4	7	51	49	0.7	4.158	1619.02307	6774	0.69
5	5	50	50	0.8	3.683	1432.18726	6900	0.70
6	3	50	50	0.9	3.244	1223.38708	6035	0.72
7	8	51	49	0.9	3.213	1233.19092	6069	0.72
8	2	52	48	0.7	4.124	1658.66748	7245	0.69

Table 1: Factor considered for the study by software used

In the final validated method, the mobile phase used is methanol and water in a 51:49 (v/v) ratio, a flow rate of 0.8 mL/min and a detection wavelength of 271 nm.

Preparation of Mobile Phase

This mobile phase was made by mixing equal volumes (50 mL each) of methanol with water containing 0.1% orthophosphoric acid which resulted in a 50:50 v/v ratio. To ensure clarity and remove any impurities, the solution was then filtered. Finally, it was sonicated for 10 minutes to promote thorough mixing and eliminate any trapped air bubbles.[6]

Preparation of Stock Solution

A standard solution with a concentration of 1000 ug/ml was prepared by dissolving 10mg of bisoprolol fumarate in 10 mL of methanol using a 10-volumetric flask.

To prepare samples for analysis, different volumes of the standard solution were taken and diluted to a final volume of 10ml with the mobile phase. This dilution process allowed for the creation of samples with varying concentrations for analysis.[7]

4. Results

Optimization

Screening design for suitable chromatographic condition

Optimization of the mobile phase, composed of methanol and water, resulted in improved peak symmetry, reduced retention time, and increased theoretical plates. Overall, the observations were highly satisfactory.

Optimized Chromatographic condition

The Mobile phase: Methanol: Water (51:49 v/v), buffer 0.1% orthophosphoric acid, flow rate 0.8ml/min.

Analytical column: C18 column ZORBAX (4.6 * 250mm * 5um), UV detection: 271 nm, run time 10 min.[8]

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Method validation

A comprehensive validation protocol was conducted following the guidelines provided by the International Conference on Harmonization (ICH) (7) to assess the performance characteristics of the proposed HPLC method. The validation process included an evaluation of the method's repeatability, specificity, precision, accuracy, and robustness, confirming its suitability for the intended purpose.

Linearity and Range

To quantify Bisoprolol Fumarate, a five-point calibration curve was constructed using independent dilutions of a stock solution, spanning concentrations from 4 to 20 µg/ml. The regression line established a strong linear relationship within this range, evidenced by a high correlation coefficient, minimal y-intercept, and well-defined slope.[9]

Table 2 Result of linearity and range study linearity and range study

Concentration	Area of Peak	SD	%RSD
4	93.46	0.01	0.01
8	178.25	0.01	0.00
12	261.53	0.00	0.00
16	342.15	0.01	0.00
20	426.95	0.01	0.00
	Average SD	0.01	

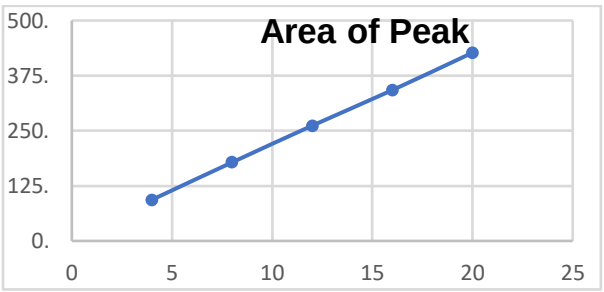

Table 3 Linearity Parameter

Parameters	HPLC Method
Range	4-20 µg /ml
Slope	20.77 m
Intercept	11.2 c
Correlation Coefficient	0.999

Repeatability

The proposed chromatographic method's repeatability was investigated by injecting a 20 µg/ml drug solution two times and evaluating the area for each injection. This ensures the system's consistent performance, vital for reliable analysis.[9]

Table 4. Result of Repeatability

Sr. no.	Concentration	Area 1	Area 2	Mean	SD	% RSD
1	20 µg /ml	2797.8558	2796.9856	2797.42	0.62	0.02

Specificity

An examination was conducted to assess the impact of excipients and additional additives commonly found in Bisoprolol Fumarate dosage forms on its determination under established optimal conditions. The formulated mobile phase exhibited effective resolution of the drug, resulting in a consistently sharp peak at 4.3±0.0098 minutes. Subsequent analysis identified 271nm as the optimal wavelength for detection, chosen for its heightened sensitivity in quantifying Bisoprolol Fumarate.

Precision

The evaluation of the demonstration involved two main aspects: the repeatability of the injection (system precision) and the precision of the method. To assess the injection repeatability, the standard bisoprolol fumarate solution was injected three times, and the %RSD of the repeated injections was calculated. To demonstrate the accuracy of the method, three samples from the same formulation batch were analyzed separately, and the assay content of each sample was determined. The mean

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Sr. no.	Accuracy	µg /ml	Amt found	Amt recovered	% Recovery
1	80%	10	121.08759	111.08755	109.00
2		10	121.12893	111.12893	108.99
3		10	121.11369	111.11825	108.99
Mean					108.98

of the repeated measurements was then calculated, along with the % RSD for the replicates.[10]

Table 8 Result of 100% extra bisoprolol fumarate standard

Sr. no.	Accuracy	µg /ml	Amt found	Amt recovered	% Recovery
1	100 %	10	135.20438	125.18331	108.00
2		10	135.18332	125.20437	107.97
3		10	135.19650	125.18989	107.99
Mean					107.98

Table 9 Result of 120% extra bisoprolol fumarate standard

Sr. no.	Accuracy	µg /ml	Amt found	Amt recovered	% Recovery
1	120%	10	147.18675	137.18675	107.28
2		10	147.22724	137.22698	107.28
3		10	147.22862	137.21591	107.29
Mean					107.28

Table 5. Result of Interday Precision

Sr. no.	Concentration	Area 1	Area 2	Mean	SD	% RSD
1	10 µg /ml	1410.2	1410.182	1401.19	00.1	0.00
2	15 µg /ml	2154.78	2153.82	11849.30	13710.12	115.70
3	20 µg /ml	2777	2777.75	2776.88	1.24	0.04

Table 6 Result of Intraday Precision

Sr. no.	Concentration	Area 1	Area 2	Mean	SD	%RSD
1	10 µg /ml	1214.967	1241.331	1241.65	0.45	0.04
2	15 µg /ml	3641.31	3640.92	3641.12	0.28	0.01
3	20 µg /ml	5933.844	5934.653	5934.25	0.57	0.01

Recovery

The proposed methodology was validated by conducting recovery studies using the standard addition technique to ensure the accuracy of the results. Previously analyzed Bisoprolol Fumarate samples (10 µg/ml) were spiked were further supplemented with bisoprolol fumarate standards at 80%, 100% and 120%. The mixtures were then analysed using the proposed method. We then calculated the standard deviation of the % recovery and mentioned below in table.[11]

The % recovery of Bisoprolol Fumarate was found to be 108.08 %

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Robustness

The robustness of a chromatographic method for analyzing a 20 µg/ml drug solution was assessed by deliberately modifying experimental conditions at three levels. Each factor, including flow rate, wavelength and temperature, was altered individually while monitoring retention time and chromatographic response. No significant influence on these parameters was observed, indicating the method's resilience to small operational adjustments, and thereby establishing its suitability for reliable drug quantification.[12]

Table 10 Result of Robustness for Flow rate 0.7ml

Sr.no.	concentration	Area	Mean	SD	%RSD
1	20 µg /ml	3041.774	3043.88	2.98	0.10
2	20 µg /ml	3045.987			

Table 11 Result of Robustness for Flow rate 0.9ml

Sr. no.	Concentration	Area	Mean	SD	%RSD
1	20 µg /ml	2450.7194	2448.07	3.75	0.15
2	20 µg /ml	2445.4152			

Table 12 Result of Robustness for wavelength 248 nm

Sr. no	Concentration	Area	Mean	SD	%RSD
1	20 µg /ml	2721.801	2723.7	2.76	0.10
2	20 µg /ml	2725.6985			

Table 13 Result of Robustness for wavelength 250 nm

Sr. no	Concentration	Area	Mean	SD	%RSD
1	20 µg /ml	2807.993	2824.39	23.19	0.82
2	20 µg /ml	2840.789			

Table 14 Result for robustness of melting point 74+26

Sr.no	Concentration	Area	Mean	SD	%RSD
1	20 µg /ml	2742.1958	2746.2	5.96	0.21
2	20 µg /ml	2750.2369			

Table 15 Result for robustness of melting 74 + 24 point

Sr.no	Concentration	Area	Mean	SD	%RSD
1	20 µg /ml	2731.7719	2732.81	1.47	0.05
2	20 µg /ml	2733.8526			

Limit of Detection and Limit of Quantification

Calculation for LOD: 3.3 * Avg SD / Slope

Calculation for LOQ: 10 * Avg SD / Slope

Table 17 LOD & LOQ

LOD	0.0003284 µg /ml
LOQ	0.0009951 µg /ml

Label Claim / Analysis of Marketed Formulation

The drug sample from tablets exhibited a peak corresponding to Bisoprolol Fumarate in the

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chromatogram. The experimental results, which indicated the percentage of Bisoprolol Fumarate in the tablets, matched closely with the information provided on the label, implying that there was no interference from the tablet excipients. The assay of drug was found to be 99.99%.[13]

Table 16 Results of Label Claim

Sr.no.	Amount found	Label claim	% Assay
1	684.60	684.61	99.99%

Force Degradation

Degradation conducted by exposing 0.5ml stock solution of bisoprolol fumarate solution to acidic conditions (0.5ml stock solution added to 2ml of 0.1 N HCl and volume make was made up to 10ml with mobile phase), basic conditions (0.5ml stock solution added to 2ml of 0.1 N NaOH and volume make was made up to 10ml with mobile phase), neutral conditions (0.5ml stock solution added to 2ml of 3% H_2O_2 and volume make was made up to 10ml with mobile phase) The Degradation of drug was seen in HCl, NaOH and is stable peak is observed that is the drug is not degraded in H_2O_2.[14]

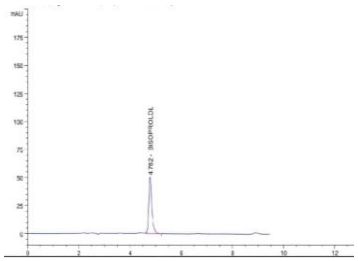

Figure 1 : Standard Chromatogram

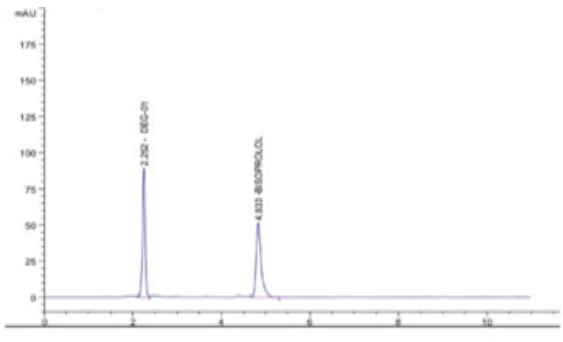

Figure 2: Force Degradation Studies - HCl (50mcg)

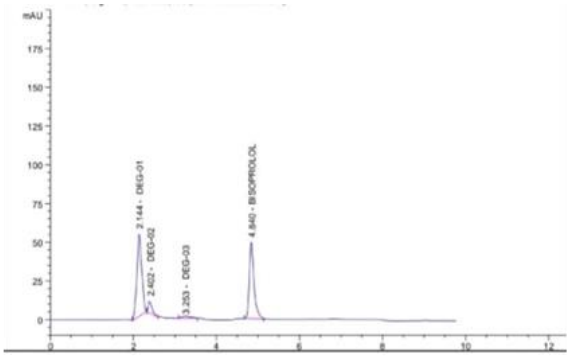

Figure 3: Force Degradation Studies - NaOH (50mcg)

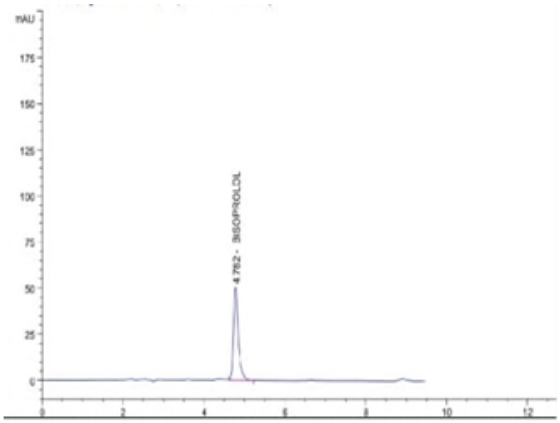

Figure 4 : Force Degradation Studies – H_2O_2 (50 mcg)

5. Discussion

A new, rapid, and highly sensitive HPLC method for determining bisoprolol fumarate in both its pure form and dosage forms is introduced in this study. This innovative approach addresses the limitations of previous methods by eliminating the need for complex isocratic elution and extended retention times. The extraction process is simplified, and readily available, cost-effective equipment, solvents, and reagents are utilized. Despite its simplicity, the method demonstrates excellent accuracy, precision, and sensitivity, making it suitable for reliable quality control applications. Overall, this novel HPLC method represents a significant advancement in the analysis of Bisoprolol Fumarate, offering a faster, more efficient, and cost-effective analytical tool for research and pharmaceutical settings.

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