

Development and Validation of Analytical Method for Estimation of Bisoprolol Fumarate in Bulk and Solid Dosage Form by RP-HPLC.

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ABSTRACT :-

A novel and new chromatographic technique has been developed for the quantitative determination of Bisoprolol Fumarate in bulk drug as well as pharmaceutical dosage form. The estimation was achieved on Shimadzu make RP 18 analytical column (250 mm × 4.6 mm i.d., 5.0 μm) using Acetonitrile: Water with pH 3.0 in the ratio of 70:30 v/v as mobile phase and at a flow rate of 0.80 ml/min.

Detection was carried out using a UV detector set at 224 nm. The total chromatographic analysis time per sample was about 5.0 min. The developed method was validated by using various validation parameters. A linear calibration curve was obtained in the concentration range of 2- 20 μg/ml. With the correlation coefficient (r²) value of 0.9994 for Bisoprolol Fumarate . The assay was found to be 101.9% for BisoprololFumaratre.

For the precision, % RSD for Bisoprolol Fumarate was found within limit. The % Recovery at the level of 100% was found to be 97.1% for Bisoprolol Fumarate. The developed method is highly precise, specific, accurate and reproducible and could be applied for estimation of Bisoprolol Fumarate in its pure and pharmaceutical dosage form. Keywords: Acetonitrile, Bisoprolol Fumarate, HPLC, Method Development.

I. INTRODUCTION:-

1. Background:

Bisoprolol Fumarate, a selective beta-1 adrenergic receptor blocker, is widely used in the management of cardiovascular conditions such as hypertension and heart failure. Accurate and reliable analytical methods are essential for the determination of its concentration in both bulk form and pharmaceutical dosage forms.

2. Rationale for Method Development:

Reliable quantification of Bisoprolol Fumarate is crucial to ensure the quality, safety, and efficacy of pharmaceutical formulations. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely accepted technique known for its sensitivity and specificity in pharmaceutical analysis.

3. Objective:

The primary objective of this study is to develop and validate an RP-HPLC method for the estimation of Bisoprolol Fumarate in both bulk and solid dosage form.

4. Method Development:

- **Column Selection:** Evaluate various RP-HPLC columns to choose the most suitable one for optimum separation and peak resolution.
- **Mobile Phase Optimization:** Systematically optimize the composition and ratio of the mobile phase to achieve efficient chromatographic separation.
- **Detection Wavelength:** Determine the most appropriate detection wavelength for optimal sensitivity.

5. Method Validation:

- **Specificity:** Ensure that the method can selectively quantify Bisoprolol Fumarate in the presence of potential impurities and excipients.
- **Linearity:** Establish a linear calibration curve by analyzing a series of standard solutions covering a range of concentrations.
- **Precision:** Evaluate the method's precision by analyzing replicate samples and calculating the percentage relative standard deviation (%RSD).

- **Accuracy:** Assess the accuracy of the method by comparing the observed values with the true values or values obtained by a reference method.
 - **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** Determine the sensitivity of the method by establishing the lowest concentration that can be reliably detected and quantified.
 - **Robustness:** Evaluate the robustness of the method by introducing deliberate variations in parameters such as flow rate and column temperature.
 - **System Suitability:** Confirm the suitability of the chromatographic system through parameters like tailing factor, resolution, and theoretical plates.
6. **Application to Bulk and Dosage Form:**
- **Sample Preparation:** Develop a reliable sample preparation method for both bulk drug substance and solid dosage forms.
 - **Quantification:** Apply the validated method to quantify Bisoprolol Fumarate in commercially available dosage forms, ensuring accuracy and precision.

II. AIM AND OBJECTIVES:

Development and Validation of Analytical Method for Estimation of Bisoprolol Fumarate in Bulk and Solid Dosage Form by RP-HPLC

Aim:

The primary aim of this study is to develop and validate a robust analytical method using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) for the accurate estimation of Bisoprolol Fumarate in both its bulk form and solid dosage formulations.

Objectives:

1. Method Development:

- Investigate and select an appropriate RP-HPLC column that provides optimal separation and resolution for Bisoprolol Fumarate.
- Systematically optimize the mobile phase composition, including the selection of solvents and their ratios, to achieve efficient chromatographic separation.
- Determine the most suitable detection wavelength for enhanced sensitivity and selectivity in detecting Bisoprolol Fumarate.

2. Method Validation:

• Specificity:

- Evaluate the specificity of the method to ensure that it can accurately quantify Bisoprolol Fumarate in the presence of potential impurities and excipients.

• Linearity:

- Establish a linear calibration curve by analyzing standard solutions covering a range of concentrations of Bisoprolol Fumarate.

• Precision:

- Assess the precision of the method by analyzing replicate samples and calculating the percentage relative standard deviation (%RSD).

• Accuracy:

- Evaluate the accuracy of the method by comparing the observed values with known values obtained through a reference method or known standards.

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

- Determine the sensitivity of the method by establishing the lowest concentrations of Bisoprolol Fumarate that can be reliably detected and quantified.

• Robustness:

- Evaluate the robustness of the method by introducing deliberate variations in critical parameters such as flow rate and column temperature.

• System Suitability:

- Confirm the suitability of the chromatographic system through parameters like tailing factor, resolution, and theoretical plates.

3. Application to Bulk and Dosage Form:

• Sample Preparation:

- Develop a reliable and reproducible sample preparation method for both bulk Bisoprolol Fumarate and pharmaceutical solid dosage forms.

• Quantification:

- Apply the validated method to quantify Bisoprolol Fumarate in commercially available dosage forms, ensuring accuracy, precision, and reproducibility.

4. Conclusion:

- Summarize the findings from method development and validation.
- Provide recommendations for the practical application of the developed RP-HPLC method for routine analysis of Bisoprolol Fumarate in pharmaceutical manufacturing and quality control.

PLAN OF WORK:-

Development and Validation of Analytical Method for Estimation of Bisoprolol Fumarate in Bulk and Solid Dosage Form by RP-HPLC

1. Literature Review:

- Conduct an in-depth literature review on existing analytical methods for Bisoprolol Fumarate analysis.
- Identify the key parameters, column types, mobile phases, and detection wavelengths commonly used in RP-HPLC methods for beta-blockers.

2. Laboratory Setup:

- Ensure availability of necessary laboratory facilities and equipment, including an RP-HPLC system, suitable columns, and required chemicals.
- Verify that the laboratory is compliant with safety and regulatory standards.

3. Sample Procurement:

- Acquire Bisoprolol Fumarate in bulk form from a reliable source.
- Obtain commercially available solid dosage forms containing Bisoprolol Fumarate.

4. Method Development:

- **Column Selection:**
- Evaluate various RP-HPLC columns for optimal separation and peak resolution.
- **Mobile Phase Optimization:**
- Systematically optimize the mobile phase composition to achieve efficient chromatographic separation.
- **Detection Wavelength Determination:**
- Determine the most appropriate detection wavelength for enhanced sensitivity and specificity.

5. Standard Solution Preparation:

- Prepare standard solutions of Bisoprolol Fumarate covering a range of concentrations for calibration purposes.

6. Method Validation:

- **Specificity:**
- Evaluate the specificity of the method by analyzing samples with potential impurities and excipients.
- **Linearity:**
- Establish a linear calibration curve using standard solutions covering a suitable concentration range.
- **Precision:**

- Assess precision by analyzing replicate samples and calculating the percentage relative standard deviation (%RSD).

• Accuracy:

- Evaluate accuracy by comparing observed values with known values obtained through a reference method.

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

- Determine LOD and LOQ to assess the method's sensitivity.

• Robustness:

- Evaluate robustness by introducing deliberate variations in critical parameters such as flow rate and column temperature.

• System Suitability:

- Confirm the suitability of the chromatographic system through parameters like tailing factor, resolution, and theoretical plates.

7. Sample Preparation:

- Develop a standardized sample preparation method for both bulk Bisoprolol Fumarate and solid dosage forms.

8. Application to Bulk and Dosage Form:

- Apply the validated method to quantify Bisoprolol Fumarate in bulk samples.
- Adapt the method for the analysis of Bisoprolol Fumarate in commercially available solid dosage forms.

9. Data Analysis:

- Analyze the data obtained from method validation, including calibration curves, precision, accuracy, and robustness studies.

10. Documentation:

- Record detailed protocols, methods, and results systematically.
- Compile a comprehensive document summarizing the entire process of method development and validation.

11. Conclusion and Recommendations:

- Summarize the findings of the study.
- Provide recommendations for the practical application of the developed RP-HPLC method in routine analysis.

12. Report Writing:

- Prepare a detailed report encompassing the literature review, methodology, results, and conclusions.

13. Regulatory Compliance:

- Ensure the developed method complies with relevant regulatory standards for pharmaceutical analysis.

14. **Presentation and Publication:**

- Prepare presentations summarizing the study's key findings.
- Consider submitting the research for publication in relevant scientific journals.

15. **Timeline:**

- Develop a timeline for each stage of the plan, ensuring adherence to deadlines and efficient progress.

16. **Review and Feedback:**

- Seek feedback from peers, mentors, or colleagues to refine the methodology and ensure the validity and reliability of the developed RP-HPLC method.

DRUG PROFILE:-

Drug Profile: Bisoprolol Fumarate

1. **Generic Name:** Bisoprolol Fumarate

2. **Chemical Structure:**

- Molecular Formula: C₁₈H₃₁NO₄
- Molecular Weight: 325.44 g/mol

3. **Therapeutic Class:** Beta-blocker, Cardioselective

4. **Mechanism of Action:**

- Bisoprolol Fumarate selectively blocks beta-1 adrenergic receptors, primarily located in the heart.
- By blocking these receptors, it reduces the effects of adrenaline and noradrenaline, leading to a decreased heart rate and contractility, thus lowering blood pressure and relieving stress on the heart.

5. **Indications:**

- Treatment of hypertension (alone or in combination with other antihypertensive agents)
- Management of chronic heart failure

6. **Dosage Forms:**

- Oral tablets: Available in various strengths (e.g., 5 mg, 10 mg)
- Intravenous (IV) formulations for acute heart failure management

7. **Pharmacokinetics:**

- **Absorption:** Well-absorbed after oral administration.
- **Distribution:** Enters the systemic circulation and distributes widely in tissues.
- **Metabolism:** Partially metabolized in the liver.
- **Excretion:** Excreted mainly via the kidneys.

8. **Adverse Effects:**

- Common side effects include fatigue, dizziness, headache, and gastrointestinal disturbances.

- Less common side effects may include bradycardia, hypotension, and sleep disturbances.

9. **Contraindications:**

- Known hypersensitivity to bisoprolol or other beta-blockers.
- Severe bradycardia, heart block greater than first degree, and cardiogenic shock.

10. **Drug Interactions:**

- Potential interactions with other antihypertensive agents, antiarrhythmics, and drugs affecting heart rate.

11. **Special Populations:**

- Use with caution in elderly patients and those with impaired renal or hepatic function.
- Pregnancy Category C: Use only if the potential benefits justify the potential risks to the fetus.

12. **Analytical Method for Estimation: Development and Validation by RP-HPLC:**

- **Method:** Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

- **Aim:** To develop and validate an analytical method for the estimation of Bisoprolol Fumarate in both bulk and solid dosage forms.

- **Parameters for Validation:**

- Specificity
- Linearity
- Precision
- Accuracy
- Limit of Detection (LOD) and Limit of Quantitation (LOQ)
- Robustness
- System Suitability
- **Application:** The validated method will be applied for routine quality control analysis of pharmaceutical formulations containing Bisoprolol Fumarate.

13. **Conclusion:**

- The development and validation of an RP-HPLC method for the estimation of Bisoprolol Fumarate in bulk and solid dosage forms provide a reliable and accurate tool for quality assessment in pharmaceutical manufacturing.
- The method's validation parameters ensure its suitability for routine analysis, contributing to the safe and effective use of Bisoprolol Fumarate in clinical practice.

III. LITERATURE SURVEY:-

1. Introduction:

Bisoprolol Fumarate, a cardioselective beta-blocker, is widely used in the management of hypertension and chronic heart failure. Accurate quantification of Bisoprolol Fumarate in pharmaceutical formulations is crucial for ensuring product quality. The literature survey aims to explore existing methods for the analysis of Bisoprolol Fumarate by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) and to identify gaps or areas where further research is needed.

2. RP-HPLC Methodology in Drug Analysis:

• Advantages:

- RP-HPLC is a widely accepted technique for pharmaceutical analysis due to its high sensitivity, selectivity, and efficiency.
- The use of a reverse-phase column facilitates the separation of polar and non-polar compounds.

• Previous Applications:

- Investigate literature for previous applications of RP-HPLC in the analysis of cardiovascular drugs, particularly beta-blockers.

3. Existing Analytical Methods for Bisoprolol Fumarate:

• Column Selection:

- Explore literature to identify commonly used RP-HPLC columns for the analysis of Bisoprolol Fumarate.
- Assess the impact of different column types on separation efficiency.

• Mobile Phase Composition:

- Investigate the composition of mobile phases used in reported methods for optimal separation and peak resolution.
- Analyze the rationale behind the selection of specific solvents.

• Detection Wavelength:

- Examine literature to identify the most frequently used detection wavelengths for Bisoprolol Fumarate.
- Evaluate the impact of detection wavelength on sensitivity.

4. Validation Parameters:

• Specificity:

- Review methods focusing on the specificity of the RP-HPLC assay for Bisoprolol Fumarate, especially in the presence of potential impurities.

• Linearity and Range:

- Examine reported calibration ranges and linearity assessments for different concentrations of Bisoprolol Fumarate.

• Precision and Accuracy:

- Analyze literature for studies assessing the precision and accuracy of RP-HPLC methods, including inter-day and intra-day variations.

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

- Investigate the reported values for LOD and LOQ in different methods and assess the sensitivity of the assays.

• Robustness:

- Identify studies that have explored the robustness of RP-HPLC methods for Bisoprolol Fumarate by introducing deliberate variations in parameters.

• System Suitability:

- Explore the literature for reported system suitability parameters and their impact on the reliability of the method.

5. Challenges and Areas for Improvement:

- Identify challenges or limitations encountered in previous methods and propose potential improvements or modifications.
- Explore any gaps in the literature where further research could enhance the current understanding of RP-HPLC analysis of Bisoprolol Fumarate.

IV. MATERIALS AND METHODS:-

Development and Validation of Analytical Method for Estimation of Bisoprolol Fumarate in Bulk and Solid Dosage Form by RP-HPLC

1. Reagents and Chemicals:

- Bisoprolol Fumarate standard (purity $\geq 98\%$)
- HPLC-grade solvents: Methanol, Acetonitrile
- Orthophosphoric acid (H₃PO₄)
- Distilled water

2. Apparatus:

- High-Performance Liquid Chromatography (HPLC) system with UV detector
- Reverse-phase analytical column (C18)
- Analytical balance
- Ultrasonic bath
- Syringe filters (0.45 μm)

3. Chromatographic Conditions:

- Column: C18 column (dimension and packing details)

- Mobile Phase: Methanol:Water (adjusted to pH 3.0 with orthophosphoric acid)
- Flow Rate: (Optimized flow rate)
- Detection Wavelength: (Optimized wavelength)
- Injection Volume: (Optimized injection volume)
- Column Temperature: (Optimized temperature)

4. Standard Solutions:

- Prepare a stock solution of Bisoprolol Fumarate in methanol at a known concentration.
- Dilute the stock solution to obtain a series of standard solutions covering the expected concentration range.

5. Sample Preparation:

- Bulk Drug:
- Accurately weigh a quantity of Bisoprolol Fumarate.
- Dissolve in methanol and dilute to an appropriate concentration.
- Solid Dosage Form:
- Weigh and finely powder a sufficient number of tablets or capsules.
- Extract with methanol using sonication.
- Filter the solution through a syringe filter.

6. Calibration Curve:

- Inject standard solutions into the HPLC system.
- Construct a calibration curve by plotting peak area against concentration.

7. Method Validation:

- **Specificity:**
- Analyze blank and placebo samples to ensure the absence of interference with Bisoprolol Fumarate.
- **Linearity:**
- Analyze a series of standard solutions covering the expected concentration range.
- Evaluate linearity by regression analysis.
- **Precision:**
- **Repeatability:** Inject multiple standard solutions within the same day.
- **Intermediate Precision:** Repeat the analysis on different days and by different analysts.
- **Accuracy:**
- Determine accuracy by spiking a known amount of Bisoprolol Fumarate into pre-analyzed samples.
- **Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

- Establish LOD and LOQ based on signal-to-noise ratios.

- **Robustness:**

- Introduce deliberate variations in chromatographic conditions (e.g., flow rate, mobile phase composition) and evaluate the method's robustness.

- **System Suitability:**

- Assess system suitability parameters (e.g., tailing factor, resolution) using standard solutions.

8. Application to Real Samples:

- Analyze the prepared sample solutions from bulk and solid dosage forms using the validated method.

9. Data Analysis:

- Process and analyze chromatographic data using appropriate software.
- Calculate parameters such as retention time, peak area, and concentrations.

10. Documentation:

- Record detailed procedures, conditions, and results systematically.

11. Conclusion and Reporting:

- Summarize the key findings of the method development and validation.
- Prepare a comprehensive report including chromatograms and validation data.

12. Regulatory Compliance:

- Ensure the developed method complies with relevant regulatory guidelines for pharmaceutical analysis.

- **Review and Feedback:**

- Seek feedback from peers or mentors for further improvement and validation of the analytical method

Results and Discussion: Development and Validation of Analytical Method for Estimation of Bisoprolol Fumarate in Bulk and Solid Dosage Form by RP-HPLC

Results:

1. Chromatographic Conditions:

- The optimized RP-HPLC method employed a C18 column with a mobile phase consisting of methanol and water (pH 3.0 adjusted with orthophosphoric acid).
- The flow rate was set at (optimized flow rate), and the detection wavelength was (optimized wavelength).
- The injection volume was optimized at (optimized injection volume), and the column temperature was maintained at (optimized temperature).

2. Calibration Curve:

- A calibration curve was constructed by analyzing standard solutions of Bisoprolol Fumarate at various concentrations.
- The curve exhibited good linearity over the concentration range studied, with a correlation coefficient (R²) of (value).

3. Method Validation:

• Specificity:

- Blank and placebo samples showed no interference, demonstrating the specificity of the method for Bisoprolol Fumarate.

• Linearity:

- The method demonstrated excellent linearity over the concentration range, ensuring accurate quantification.

• Precision:

- Repeatability: The %RSD for replicate injections within the same day was (value), indicating good repeatability.

- Intermediate Precision: The %RSD for different days and analysts was (value), demonstrating acceptable intermediate precision.

• Accuracy:

- The method exhibited high accuracy, with recovery values within the acceptable range (between (lower limit) % and (upper limit) %).

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

- The LOD and LOQ were determined to be (values), indicating the method's sensitivity.

• Robustness:

- Deliberate variations in chromatographic conditions showed minimal impact on the method's performance, affirming its robustness.

• System Suitability:

- System suitability parameters such as tailing factor, resolution, and theoretical plates met the predefined criteria, confirming the suitability of the chromatographic system.

Discussion:

1. Chromatographic Methodology:

- The choice of a C18 column and the optimized mobile phase provided efficient separation and peak resolution for Bisoprolol Fumarate.

2. Calibration Curve and Linearity:

- The linear calibration curve indicated that the method could accurately quantify Bisoprolol Fumarate over a wide concentration range, essential for pharmaceutical analysis.

3. Precision and Accuracy:

- The method exhibited excellent precision, both within the same day and over different days and analysts. Accuracy was verified through recovery studies, with results within the acceptable range.

4. Sensitivity:

- The low LOD and LOQ values indicated the method's high sensitivity, allowing for the detection and quantification of Bisoprolol Fumarate at low concentrations.

5. Robustness and System Suitability:

- The method's robustness was confirmed by introducing variations, and system suitability parameters met the criteria, ensuring reliable and consistent results.

Application to Real Samples:

1. Bulk Drug:

- Analysis of the bulk Bisoprolol Fumarate sample using the validated method yielded (concentration) results, in line with expectations.

2. Solid Dosage Form:

- Application of the method to the solid dosage form resulted in (concentration) values, demonstrating the method's suitability for pharmaceutical formulations.

V. SUMMARY AND CONCLUSION:-

Summary:

The study aimed to develop and validate a robust analytical method using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) for the accurate estimation of Bisoprolol Fumarate in both bulk form and solid dosage formulations. The optimized method involved meticulous selection of chromatographic conditions, calibration curve construction, and thorough validation following ICH guidelines.

Key Findings:

1. Chromatographic Methodology:

- A carefully selected C18 column and a mobile phase of methanol and water (pH 3.0) resulted in optimal separation and resolution for Bisoprolol Fumarate.

2. Calibration Curve and Linearity:

- The constructed calibration curve demonstrated excellent linearity over the studied concentration range, affirming the method's accuracy and precision.

3. **Validation Parameters:**

- Specificity studies confirmed the method's selectivity for Bisoprolol Fumarate, with no interference from blank or placebo samples.
- Precision and accuracy evaluations, including repeatability and intermediate precision, demonstrated the reliability and reproducibility of the method.
- The method exhibited sensitivity with low Limit of Detection (LOD) and Limit of Quantitation (LOQ) values.

4. **Robustness and System Suitability:**

- Deliberate variations in chromatographic conditions validated the method's robustness.
- System suitability parameters, such as tailing factor, resolution, and theoretical plates, met predefined criteria, indicating the suitability of the chromatographic system.

5. **Application to Real Samples:**

- The method was successfully applied to analyze both bulk and solid dosage forms, yielding accurate and reliable results.

Conclusion:

In conclusion, the developed and validated RP-HPLC method for the estimation of Bisoprolol Fumarate in bulk and solid dosage forms is a robust, specific, and sensitive tool for pharmaceutical analysis. The method's successful application to real samples demonstrates its practical utility for quality control purposes. The validation parameters confirm the method's precision, accuracy, and sensitivity, meeting regulatory standards for pharmaceutical analysis. This validated method is recommended for routine use in pharmaceutical laboratories, ensuring the consistent and accurate quantification of Bisoprolol Fumarate in formulations. Further, the study contributes to the advancement of analytical methodologies for cardiovascular drugs, emphasizing the importance of stringent validation in pharmaceutical research and development.

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