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A Research Review Article on Analytical Metohd Validation for Uv Spectroscopy And HPLC Methods of Bisoprolol Fumarate

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ABSTRACT
The validation of analytical methods for Bisoprolol Fumarate, a widely used beta-blocker for managing hypertension and heart failure, is crucial to ensure the drug's quality and efficacy. This study focuses on validating two key analytical techniques: Ultraviolet (UV) spectrophotometry and High-Performance Liquid Chromatography (HPLC). The research aims to assess the performance, reliability, and accuracy of these methods for Bisoprolol Fumarate quantification.The UV spectrophotometric method involves the determination of Bisoprolol Fumarate's absorbance at a specific wavelength. The study optimized parameters such as the wavelength of maximum absorbance and concentration ranges. Validation included assessing linearity, accuracy, precision, and limit of detection (LOD) and quantitation (LOQ). The method showed excellent linearity with a correlation coefficient (R^2) exceeding 0.999, indicating a high degree of accuracy. Precision studies demonstrated low relative standard deviations (RSD), ensuring repeatability. Accuracy was confirmed through recovery experiments, which yielded results within the acceptable range. The LOD and LOQ values were found to be sufficiently low, confirming the method's sensitivity for detecting Bisoprolol Fumarate.The HPLC method involved developing a chromatographic system with appropriate column, mobile phase, and detection conditions. The study used a C18 column with a mobile phase consisting of a mixture of acetonitrile and phosphate buffer, optimized for separation and peak resolution. The detection was carried out at 220 nm. Method validation focused on parameters such as specificity, linearity, precision, accuracy, robustness, and system suitability. Specificity was ensured through the absence of interference from excipients and degradation products. The method demonstrated linearity with a correlation coefficient of 0.9995, and precision was confirmed with RSD values below 1% for intra-day and inter-day analyses. Accuracy was validated through recovery studies, yielding results close to the theoretical values. Robustness testing indicated that slight variations in method parameters did not significantly affect the results, demonstrating the method's reliability under varied conditions. The HPLC method also achieved low LOD and LOQ, ensuring its suitability for routine analysis.

Keywords: - Bisoprolol Fumarate, Hypertension, UV Spectroscopy, HPLC, Analytical Method Validation

I. INTRODUCTION [1-5]

Bisoprolol Fumarate is a selective beta-1 adrenergic blocker widely utilized in the management of cardiovascular diseases, particularly hypertension and chronic heart failure. As a member of the beta-blocker class, Bisoprolol Fumarate is specifically designed to target beta-1 adrenergic receptors predominantly found in the heart. This selective action helps mitigate adverse effects commonly associated with non-selective beta-blockers, such as bronchoconstriction or peripheral vascular issues.

Chemically, Bisoprolol Fumarate is the fumarate salt form of Bisoprolol, which enhances the drug's stability and solubility, thereby improving its bioavailability and therapeutic efficacy. Bisoprolol works by competitively blocking beta-1 receptors in the myocardium, resulting in decreased heart rate, reduced myocardial contractility, and lower blood pressure. This mechanism of action makes it effective in reducing cardiac output and managing the symptoms associated with heart failure, such as dyspnea and fatigue. Additionally, Bisoprolol Fumarate is employed in controlling high blood

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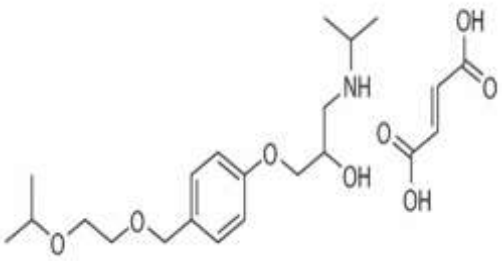
pressure, which is crucial for preventing severe cardiovascular events like stroke and myocardial infraction.



Chemical structure of Bisoprolol Fumarate

For the quantitative determination of bisoprolol fumarate in tablets – HPLC/ UV. Chromatographic conditions for the determination of drug of Bisoprolol Fumarate, tablets are given in the monograph of the United States Pharmacopoeia, where chromatographic column of L7 category and mobile phase consisting of three components: heptafluorobutyric acid, diethylamine, formic acid are used. Solvent – a mixture of water and acetonitrile (65:35), mobile phase rate – 1 ml/min, detection of wavelength – 273 nm. The proposed method of the United States Pharmacopoeia requires long sample preparation. The European Pharmacopoeia (European Pharmacopoeia 2016) has a monograph on the substance of bisoprolol fumarate. Identification of bisoprolol fumarate EPh regulates to perform the absorption spectrophotometry in the infrared region and the quantitative determination – acidimetry non-aqueous titration.

Objectives: -

- Identify appropriate indications for bisoprolol therapy, including hypertension, heart failure, and certain arrhythmias.
- Screen patients for contraindications and risk factors before initiating bisoprolol treatment, such as bradycardia, heart block, asthma, or severe peripheral vascular disease.
- Assess patients response to bisoprolol therapy, monitoring blood pressure, heart rate, and signs of adverse effects.
- Apply evidence-based guidelines and clinical recommendations to optimize bisoprolol therapy for individual patients.

❖ **Drug Profileof Bisoprolol Fumarate :-**

SR NO.	NAME	BISOPROLOL FUMARATE
1.	Molecular Formula	C18H31NO4
2.	Molecular Weight	325.45 g/mol
3.	Drug Class	Beta-blocker
4.	Mechanism of Action	Selectively inhibits beta-1 adrenergic receptors in the heart, reducing heart rate, cardiac output, and blood pressure
5.	Route of Administration	Oral
6.	Dosage Forms	Tablets
7.	Administration Frequency	Once in Daily
8.	Half-Life	Approximately 9-12 hours
9.	pKa	9.5
10.	Log P	1.3
11.	Melting point	133-136 °C
12.	Common Dosages	5 mg, 10 mg (typical starting dose: 5 mg/day)
13.	Indications	Hypertension, Chronic Heart Failure, Angina Pectoris
14.	Metabolism	Metabolized primarily in the liver by cytochrome P450 enzymes
15.	Excretion	Mainly via the kidneys (urine)
16.	Side Effects	Fatigue, dizziness, bradycardia, hypotension, cold extremities, gastrointestinal disturbances
17.	Contraindications	Asthma, severe bradycardia, certain types of heart block, severe liver impairment
18.	Drug Interactions	May interact with other antihypertensives, certain

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		antiarrhythmics, and drugs affecting hepatic metabolism
19.	Special Precautions	Caution in patients with diabetes, thyroid disorders, and peripheral vascular disease

MECHANISM OF ACTION[6-10]

- Drugs that selectively inhibit the B1 receptor have adverse inotropic and chronotropic effects; they lower heart rate and cardiac contractions. Overall, myocardial cells use less oxygen when exposed to bisoprolol. The juxtaglomerular cells have B1 receptors as well. Bisoprolol inhibits these receptors, which lowers renin release. This renin reduction prevents the renin-angiotensin system from being activated.
- Both juxtaglomerular cells and cardiac myocyte cells include B1 adrenergic receptors. When either norepinephrine or circulating catecholamines are present, they couple with the G-stimulatory protein receptor (Gs receptor) and get activated. Increased heart rate, contraction, and myocyte contraction strength are the end results of activating B1 receptors in cardiac myocytes, which also has favorable chronotropic and inotropic effects (via the exchange of GTP to GDP). In the end, this process raises the concentration of intracellular calcium and stimulates cardiac cell contraction.
- Renin-angiotensin system activation results from B1 receptor activation on juxtaglomerular cells. An angiotensin-converting enzyme (ACE) eventually transforms angiotensin I produced in response to renin release into angiotensin II.
- Numerous bodily organs contain B2 receptors, which are activated by adrenaline and produce varying symptoms depending on the location. It counteracts the impact of alpha-1 receptors, which cause vasoconstriction in the peripheral arteries, by causing vasodilation and decreasing peripheral resistance. Significant bronchodilation occurs on the bronchioles as a result. Furthermore, the activation of B2 receptors in the muscles and liver triggers the production of glucagon and glycogenolysis, which raises blood sugar levels.
- Drugs known as non-selective beta-blockers inhibit the B1 and B2 receptors, lowering cardiac output and renal renin release. Furthermore, vasoconstriction of the peripheral arteries is one of the extra signs caused by B2 receptor blocking. When B2 receptors are blocked, the lung's bronchial muscles constrict, which can induce bronchospasm in people with asthma or COPD. Hypoglycaemia may result from its decreased glucagon release and glycogenolysis.

DOSE AND ADMINISTRATION

For Adults:

Hypertension: Start with 5 mg once daily. The dose can be increased to a maximum of 10 mg daily, depending on the patient's response and tolerance.

Heart Failure: Begin with 1.25 mg once daily. The dose is gradually increased, with the maximum being 10 mg daily, based on clinical response and tolerance.

For Elderly Patients:

For Hypertension: Begin with 2.5 mg, with adjustments made based on tolerance and response.

For Heart Failure: starting doses may also be lower, with gradual increases.

II. MATERIALS AND METHODS:[11-14]

Chemical, solvent and Reagents:

- The pharmaceutical grade Bisoprolol Fumarate is purchased from MSPL. The solvent use for procedure is analytical grade. The HPLC grade chemical used is Acetonitrile and double distilled water and they were obtained from Finar Ltd & Ortho-phosphoric acid obtained from Merck Ltd. All the solvents used for HPLC such as water, acetonitrile & Ortho-phosphoric acid were of HPLC grade which were initially sonicated for 5 minutes and then filtered through membrane filter to remove any particulate present.
- **Apparatus:** U.V. Visible double beam spectrophotometer Shimadzu along with two matched cuvettes was used. Stock solutions of the samples were prepared in AR grade Acetonitrile and used for analysis. The HPLC system used is the water HPLC model LC 20 AD. The column used was C-18 (250 x 4.6 mm, 5µ). The auto sampler SIL-20AC HT with capacity 0.1 µL to 100 µL. Software used for UHPLC is Version DB 6.110 Lab Solution.

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Chromatographic conditions:

The estimation was achieved on Shimadzu make RP 18 analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using Acetonitrile: Water pH 3.0 in the ratio 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.

Preparation of sample solution: Water pH 3.0 adjusted using ortho-phosphoric acid It is filtered through 0.45 µ filter paper & sonicated for 5 min.

Mobile Phase: Mixed 700 ml of Acetonitrile, 300ml of water at pH 3.0 filtered through 0.45 µ Filter paper and sonicated for 5 min.

Solution preparation: Weighed 100 mg of Bisoprolol Fumarate and dissolved it in 100 ml of mobile phase – Stock solution (1000 µg/ml) :1 ml of Stock solution diluted to 100 ml with mobile phase. (1 µg/ml)

UV VISIBLE SPECTROSCOPY[15-18]

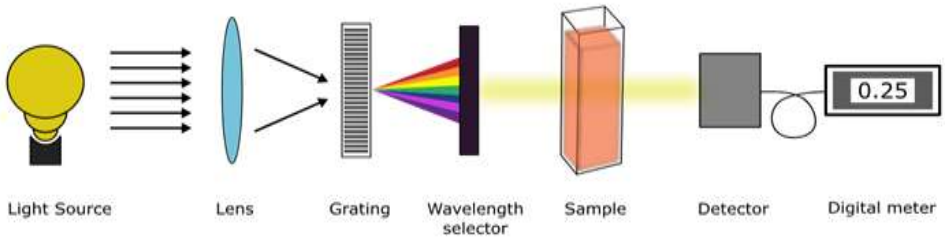
SPECTROSCOPY: -

- Spectroscopy is a branch of science dealing with the study of interactions of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta.
- Ultraviolet (UV) spectroscopy is a physical technique of optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges and it is based on **Beer-Lambert law** states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. Thus, a fixed path length can be used to determine the concentration of the absorber in a solution.
- **Beer-Lambert law: -** When a beam of light is passed through a transparent cell containing a solution of an absorbing substance, a reduction of the intensity of light may occur. Mathematically, Beer-Lambert law is expressed as

A=a b c

Where, A=absorbance or optical density,
a=absorptivity or extinction coefficient,
b=path length of radiation through the sample (cm),
c=concentration of solute in solution.

INSTRUMENTATION OF UV SPECTROPHOTOMETER:-



Single beam spectrophotometer

1. Sources (UV and visible) :- Provides illumination at one or more specific wavelengths.
2. Filter or Monochromator :- Used to select the wavelength of light that passes through the sample.
3. Sample cells :- A sample cell in UV spectroscopy is a container that holds a sample for measurement. Sample cells are made from materials that transmit UV or visible light, such as quartz, glass, or plastic. The type of cell used depends on the wavelength of light being measured and the strength of the absorption.
4. Detector :- Measures the intensity of the light that passes through the sample.
5. Readout device :- Records the absorbance or transmission of light at each wavelength.

Methods :-

- There are **two main methods** for performing UV-Vis spectroscopy: **absorption spectroscopy and transmission spectroscopy**.
- In absorption spectroscopy, a sample is placed in the path of light and the absorbance of light at each wavelength is measured.
- In transmission spectroscopy, the sample is placed in a cuvette and the transmitted light at each wavelength is measured.

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Application:-

- UV-Vis spectroscopy is used in a variety of applications, including analytical chemistry, biochemistry, environmental science, and pharmaceuticals.
- It can be used to identify and characterize molecules, measure the concentration of molecules in solution, and determine the purity of a sample.
- UV-Vis spectroscopy is a powerful analytical tool that can provide information about the structure, function, and dynamics of molecules.
- UV-Vis spectroscopy can be used to monitor the progress of a reaction and to detect impurities in a sample.
- By measuring the absorbance or transmission of light at specific wavelengths, UV-Vis spectroscopy can be used to identify and characterise molecules.
- UV-Vis spectroscopy can be used to measure the concentration of molecules in solution and to determine the purity of a sample.

HPLC METHOD[19-33]

PRINCIPLE OF HPLC: -

- A separation column separates the stationary and mobile phases during purification.
- In a separation column, the stationary phase is a granular substance with very small porous particles.
- The mobile phase is a solvent or solvent combination that is pushed through the separation column under high pressure.
- The sample is loaded into the mobile flow regime from the pump to the separation column using a syringe through a valve with a linked sample loop, i.e. a tiny tube or capillary made of stainless steel.
- A chromatogram is generated in the HPLC software at the conclusion of this operation/run.
- The chromatogram allows the various compounds to be identified and quantified.
- As a result, owing to interactions with the stationary phase, the constituent components of a mixture migrate through the column at different speeds.
- Individual compounds are identified by an appropriate detector after exiting the column and transmitted as a signal to the computer's HPLC software.

Types of HPLC

1. Normal Phase HPLC

- They are also known as normal-phase or absorption chromatography. This method separates analytes based on polarity.
- It has a polar stationary phase and a non-polar mobile phase.
- Therefore, the stationary phase is usually silica, and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures.
- The technique is used for water-sensitive compounds, geometric isomers, cis-trans isomers, class separations, and chiral compounds.

2. Reverse Phase HPLC

- The stationary phase is nonpolar (hydrophobic), while the mobile phase is an aqueous, moderate polar.
- It works on the principle of hydrophobic interactions; hence the more nonpolar the material is, the longer it will be retained.
- This technique is used for non-polar, polar, ionizable, and ionic molecules.

3. Size-exclusion HPLC

- It is also known as gel permeation chromatography or gel filtration chromatography.
- The column is filled with a material having precisely controlled pore sizes, and the particles are separated according to their molecular size.
- Larger molecules are rapidly washed through the column; smaller molecules penetrate the porous packing particles and elute later.
- Size-exclusion chromatography is also helpful in determining the tertiary and quaternary structure of proteins and amino acids.
- It is also used for the determination of the molecular weight of polysaccharides.

4. Ion-Exchange HPLC

- In this type of chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase.
- Same charged ions are excluded.
- This technique is used in purifying water, Ligand and Ion-exchange chromatography of proteins, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc.

5. Bio-affinity HPLC

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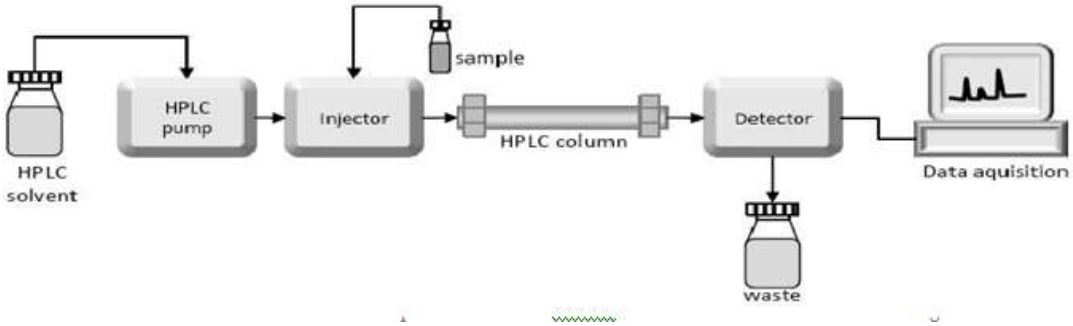
- In this type of chromatography, separation is based on the reversible interaction of proteins with ligands.

INSTRUMENTATION OF HPLC

The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The parts include:

- **Solvent Reservoir:** The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.
- **Pump:** The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector. 42000 KPa is the operating pressure of the pump. This operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.
- **Sample Injector**: The injector can be a solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 mL to 100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
- **Columns**: Columns are typically made of cleaned stainless steel, are somewhere around 50 mm and 300 mm long and have an inward distance across of somewhere around 2 and 5 mm. They are generally loaded with a stationary phase with a molecule size of 3 µm to 10 µm. Columns with inner diameters of <2 mm are regularly alluded to as microbore segments. Preferably the temperature of the mobile phase and the column should be kept consistent during investigation.
- **Detector**: The HPLC detector, situated toward the end of the column distinguishes the analytes as they elute from the chromatographic column. Regularly utilized detectors are UV-spectroscopy, fluorescence, mass spectrometric and electrochemical identifiers.
- **Data Collection Devices or Integrator**: Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The PC coordinates the reaction of the indicator to every part and places it into a chromatograph that is anything but difficult to interpret.



- **HPLC METHOD DEVELOPMENT**

A step involved in method development of HPLC is as follows:

1. Understanding the Physicochemical properties of drug molecule.
2. Selection of chromatographic conditions.
3. Developing the approach of analysis.
4. Sample preparations
5. Method optimization

- For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase. The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. pH and pKa plays an important role in HPLC method development. The pH value is defined as the negative of the logarithm to base 10 of the concentration of the hydrogen ion.

$$pH = - \log_{10}[H_3O^+].$$

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Uses of HPLC

- Purification of water.
- Impurity detection in the pharmaceutical industry.
- Trace components are pre-concentrated.
- Chromatography based on ligand exchange.
- Protein chromatography via ion exchange.
- Carbohydrate and oligosaccharide anion-exchange chromatography at high pH.

Applications of HPLC

- Drug evaluation
- Synthetic polymer analysis
- Pollution analysis in environmental analytics
- Drug determination in biological matrices
- Isolation of high-value goods

Validation Parameterfor Bisoprolol Fumarate[34-38]

Validate the developed method according to regulatory guidelines such as the International Conference on Harmonization (ICH) guidelines or specific requirements of the intended application. Validation parameters may include Specificity, Linearity, Accuracy, Precision, Robustness, Limit Of Detection (LOD), And Limit Of Quantification (LOQ), Assay.

1. Assay:

An accurately weighed amount of the powder equivalent to 100 mg of Bisoprolol Fumarate was taken and transferred into a 100 ml volumetric flask; mobile phase was added and sonicated with occasional shaking for 10 min.The solution was diluted to volume with the mobile phase. The resultant solution was filtered through 0.22 µl syringe filter. 1 ml of this solution was diluted to 100 ml with mobile phase. The final solution was filtered through membrane filter. 20µl volume of final sample solution was injected in duplicate into HPLC and peak areas were measured under optimized chromatographic conditions.

2. Limit of detection (LOD) and Limit of quantification (LOQ):

The term LOD is the lowest concentration which can be detected. The term LOQ is the lowest concentration which can be quantified. It is calculated by using the equation, LOQ = 10 × sy/S.
where "sy" represents the residual standard deviation of the regression line
"S" represent the slope of the calibration curve.

3. Precision:

Intra-day precision: To study intra-day precision, three replicate standard solutions were prepared and injected in HPLC. The results were recorded in a single day.

Inter-day precision: To study inter-day precision, three replicate standard solutions (same which were used for intra-day precision) were taken and injected into HPLC. The results were recorded for 3 consecutive days. Peak area was determined and %RSD was determined.

4. Accuracy:

Accuracy was conducted by analysing sample solution spiked with known amounts of the bulk drug or standard at three kinds of concentration levels of 50%, 100% and 150% of each at a specified limit. % recovery test was performed at all the three levels.

5. Linearity:

Linearity was studied by diluting the volume of standard stock solution (1000 µg/ml) equivalent to 0.2, 0.6, 1.0, 1.2, 1.6, 2.0 ml to 100ml with same composition of mobile phase to obtain 2, 6, 10, 12, 16, 20 µg/ml working solutions respectively.From the results obtained, calibration curve was plotted by taking concentration on X axis and mean area on Y axis.From the calibration curve drawn between concentration versus mean area, the equation of straight line, slope, intercept and regression coefficient was determined. The equation line resulted was as given herein below.

Where, Y =area of chromatogram;
$y = 11762x + 2855.7$
$R^2 = 0.9994$

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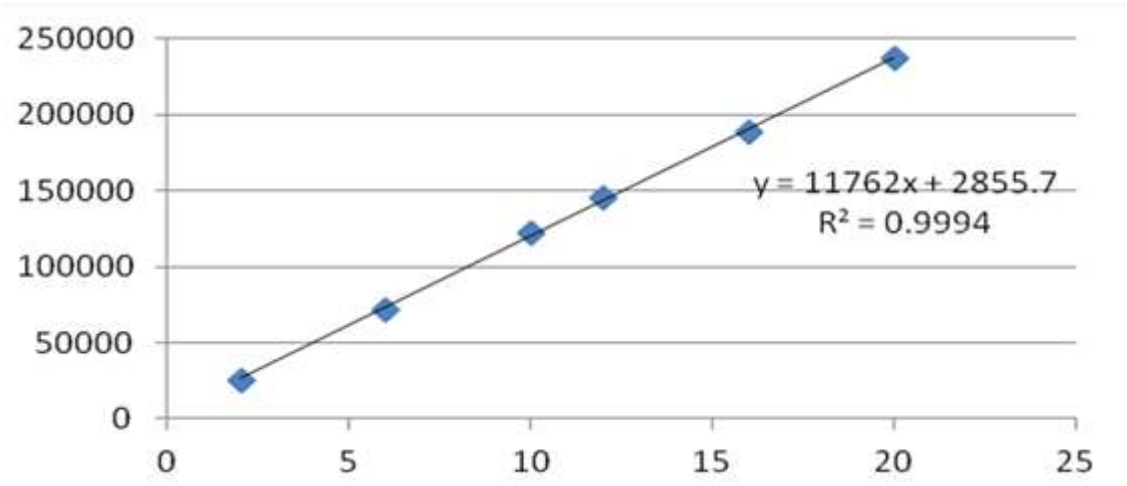

Calibration Curve of Bisoprolol Fumarate

6. Specificity and selectivity:

Specificity and selectivity were studied for the examination of the presence of interfering components in the working solution of Bisoprolol Fumarate. The results indicate that the retention time of Bisoprolol Fumarate is at about 2.946 minutes. There is no variation in the retention time of the compound as compared to the standard drug and free from interference from formulation excipient and solvent. This indicates that the method found selective and specific for the determination of Bisoprolol Fumarate.

7. Range

The range of the method is the interval between the upper and lower levels of an analyte that have been determined with acceptable precision, accuracy and linearity. It is determined on either a linear or nonlinear response curve (ie where more than one range is involved, as shown below) and is normally expressed in the same units as the test results.

If analytical procedure provides suitable level of accuracy, linearity and precision after applied to component having amounts of analyte within or at the extremity of the specified range of the analytical procedure, the range can be established.

Minimum specified range which is to be considered is as:

- Assay for drug substance or drug product: 80-120% of concentration of test.
- Content Uniformity: 70-130% of the concentration of test,
- Dissolution Testing: +/-20 % over the range which is specified.

When assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities to 120% of the assay specification.

8. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

During development phase, robustness evaluation is generally considered. It depends on the type of procedure used. By deliberate variations in the parameters, reliability of the analysis should be obtained. The conditions for the analysis should be controlled when the measurements are sensitive to the analytical condition variation.

Robustness can also be found out by establishing system suitability parameters like resolution which helps to certain that whenever the analytical procedure is used its validity is maintained. A statement of procedure should also be provided into the procedure.

Variations which usually involved that may affect the results of analysis are as follows:

- pH variation
- Stability of solutions used in analysis
- Extraction time
- Mobile phase concentration variation
- pH variation in the mobile phase
- Variation in Flow Rate
- Composition of mobile phase
- Variation in columns

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- Variation in Column size
- Variation in Injection Volume
- Variation in Temperature

❖ **UV Spectrophotometric method of Bisoprolol Fumarate**

Sr no.	Title	Description	Ref.
1.	Development and Validation of UV Spectrophotometric Method for Determination of Bisoprolol Fumarate in Bulk and Pharmaceutical Dosage Forms	Solvent: Methanol λmax: 271nm Linearity: 5 - 30µg/ml	[39]
2.	Development And Validation of Uv Spectrophotometric Method For The Determination Of Bisoprolol In Bulk Material And In Tablets	Solvent: Acetonitrileand Methanol λmax: 273 nm Linearity: 10-60 µg/ml	[40]
3.	New Validated UV Spectrophotometric Method for the Quantification of Bisoprolol Fumarate in its Pharmaceutical Dosage Form	Solvent: Phosphate buffer and Methanol λmax: 268 nm Linearity: 10-60 µg/ml	[41]

❖ **HPLC method of Bisoprolol Fumarate**

Sr no.	Title	Description	Ref
1.	Quantitative Determination of Bisoprolol Fumarate by HPLC	Mobile phase: mixture of water / methanol / acetonitrile in a ratio of 50:30:20 (v/v/v) Stationary phase: Eclipse XDB C18 type column Flow rate: 1 ml/min. λmax: 225nm Linearity: 80 - 1000 g/mL Retention time: 1.47 min	[42]
2.	Development and Validation of a Simultaneous HPLC Method for Estimation of Bisoprolol Fumarate and Amlodipine Besylate from Tablets	Mobile phase: ammonium acetate and methanol (65: 35) Stationary phase:Luna C18-2 column (3 µ, 50×4.6 mm ID) Flow rate: 0.8 ml/min λmax: 230 nm Linearity: 8–33 µg/ml Retention time: 1.45min	[43]
3.	Development and Validation of Stability Indicating Rp-Hplc Method for the Estimation of Bisoprolol Fumarate in Bulk and Pharmaceutical Dosage Form	Mobile phase: Acetonitrile: Water (60:40)v/v Stationary phase:sunsil C18 (150mm X 4.6mm, 5µ)column Flow rate: 0.8 ml/min λmax: 232 nm Linearity: 4–14 µg/ml Retention time: 1.990 min	[44]

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4.	RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation	Mobile Phase:Potassium Hydrogen Phosphate Buffer :Acetonitrile (70:30, V/V) Stationary Phase:Inertsil ODS 3V (25 Cm × 4.6 Mm) 5 mm Column Flow Rate: 1.0 ml/Min λmax: 228 nm Linearity: 2.5–50 mg/ml Retention Time: 1.75 Min	[45]
5.	Development and validation of a fast and simple HPLC method for the simultaneous determination of bisoprolol and enalapril in dosage form	Mobile Phase:55%Methanol and 45% Perchloric Acid (0.07%V/V). Stationary Phase:Zorbax Rx C8 250x4.6mm, 5um Column Flow Rate: 1.0 ml/min λmax: 273 nm Linearity: 20–200 mg/ml Retention Time: 1.75 min	[46]
6.	Stability-indicating RP-HPLC method development and validation for simultaneous estimation of bisoprolol fumarate and amlodipine besylate in bulk and in tablet dosage form	Mobile Phase: Orthophosphoric Acid: Methanol: Acetonitrile (42:29:29, V/V/V) Stationary Phase:Oyster ODS3 (150 × 4.6 Mm, 5 µm) Column Flow Rate: 1.0 ml/min λmax: 230 nm Linearity: 50–150 mg/ml Retention Time: 2.543 min	[47]
7.	DEVELOPMENT AND VALIDATION OF HPLC-DAD METHOD FOR THE DETERMINATION OF BISOPROLOL IN TABLET DOSAGE FORMS	Mobile Phase:Acetonitrile: Phosphate Buffer (25:75 V/V) Stationary Phase:Waters Symmetry C18 Column Flow Rate: 1.4 ml/min λmax: 226 nm Linearity: 70–140 mg/ml Retention Time: 2.09 min	[48]
8.	DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF BISOPROLOL FUMARATE IN BULK AND SOLIDE DOSAGE FORM BY RP-HPLC	Mobile Phase:Acetonitrile: Water 70:30 V/V Stationary Phase: RP 18 Analytical Column Flow Rate: 0.80ml/min λmax: 224 nm Linearity: 0–25 mg/ml Retention Time: 2.946 min	[49]

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9.	DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF BISOPROLOL FUMARATE TABLETS	Mobile Phase: Buffer (Ph 5.6) And Acetonitrile 750:250 Stationary Phase: C18, (250X4.6) Mm, 5µ Column Flow Rate: 1.00ml/min λmax: 226 nm Linearity: 25–100 mg/ml Retention Time: 9.15 min	[50]

III. CONCLUSION

- Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Bisoprolol Fumarate in API and tablet dosage forms. Both methods are suitable for the determination of Bisoprolol Fumarate in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations.
- The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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