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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF BISOPROLOL FUMARATE IN BULK AND SOLIDE 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 Bisoprolol Fumaratre. For the precision, % RSD for Bisoprolol Fumarate was found with in 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 Developement

Introduction:

When a novel pharmaceutical product is discovered, developed, or manufactured, analytical method development is necessary. Chromatography is mostly used for product analysis. The separation, identification, purification, and quantification of numerous compounds for study in the pharmaceutical industry as well as other industries including cosmetics, food, and energy are all commonly done using the HPLC technology. For analytical purposes, reverse phase HPLC (RP-HPLC) is most frequently utilized.

Hypertension is a complex disease, with numerous cardiovascular complications and beyond, causing a high mortality rate. The most potent beta1selective beta-blocker is bisoprolol fumarate. It has the highest level of selective beta1activity, and by inhibiting beta1adrenergic receptors, it decreases blood pressure by slowing down the contraction of the heart. Bisoprolol fumarate is known chemically as 1-[(propan2-yl) amino].-3-(4-

{[2-(propan-2 yloxy)ethoxy]methyl}phenoxy)propan-2-ol. Bisoprolol fumarate has the chemical formula C18H31NO4. The FDA granted approval on July 7, 1992.

MATERIALS AND METHODS:

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 form 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.

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)

Analytical method development :

Analytical method was developed by considering various parameters such as, mobile phase ratio, flow rate, pH, etc. Various mobile phase combinations and ratios were tried to check the maximum response. Suitable method development is essential when talking about cost, time, productivity, and effectiveness of drug product. Analytical method development is essential for drug degradation studies, analyzing and evaluating properties of API, and to study impurities in the drug.

Method validation:

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:

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.

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

The term LOD is the lowest concentration which can be detected. It is calculated by using the equation, LOD = 3.3 × sy/S. 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 and "S" represent the slope of the calibration curve.

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.

Accuracy:

Accuracy was conducted by analyzing 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.

Result and Discussion:

Analytical method development :

A reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of Bisoprolol fumarate API. 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. Bisoprolol Fumarate show two peaks in a chromatogram, one eluted first was that of Fumaric acid, which was not considered for validation and later on peak of Bisoprolol got eluted, which was considered as peak of interest. Typical Chromatogram of Bisoprolol working standard showing two peaks-

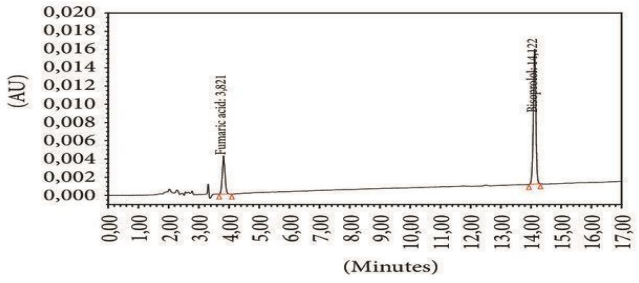


Fig 1:HPLC chromatogram of Bisoprolol Fumarate

Method Validation :

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. All of these solutions were injected to aforesaid chromatographic conditions to quantitatively estimate the area and equivalent concentration. 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

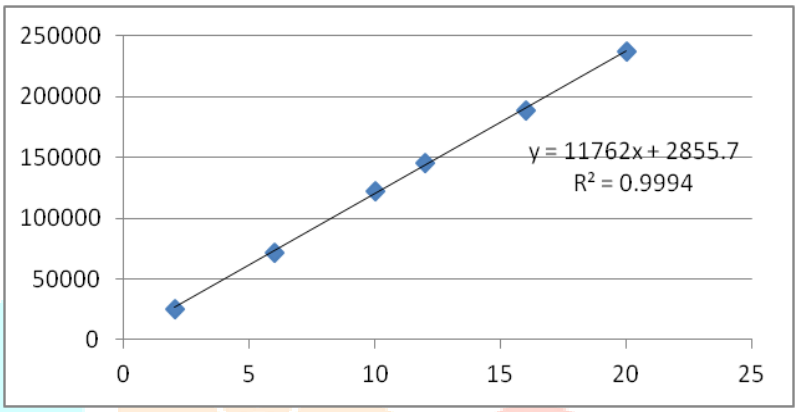


Fig 2: Calibration Curve of Bidoprolol Fumarate

Level	Conc. in µg /ml	Area
I	2	25574
II	6	72006
III	10	122924
IV	12	146096
V	16	189307
VI	20	237499

Table 01: summary of linearity

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.

Assay: Assay was determined as % deug content. The % content for Bisoprolol Fumarate was found to be 101.9 % . All calculated values are mentioned as follows

Fofmula for % Assay =

$$\frac{\text{Area of sample soln x conc. of std x purity x 100}}{\text{Area of standard soln x conc. of sample x 100}}$$

$$\% \text{ Assay} = \frac{124143 \times 10 \times 100 \times 100}{121810 \times 10 \times 100} = 101.9\%$$

Injection 1:

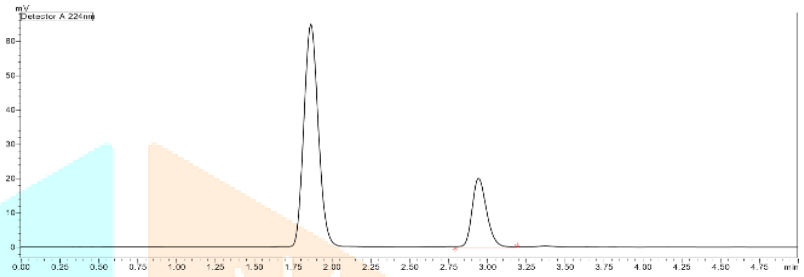
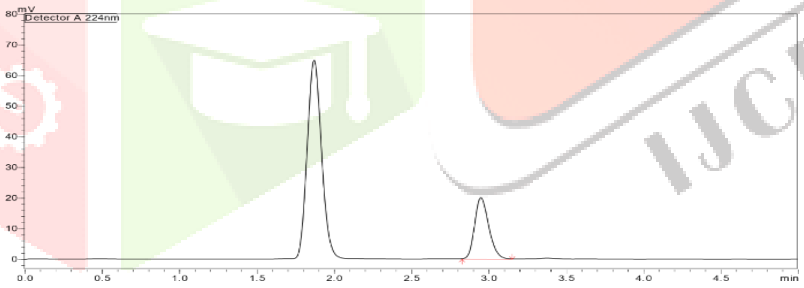


Fig 03: Assay of tablet Injection

Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
2.946	124012	100.000	1.240	4363

Table 02: summary of injection 1

Injection 2 :



Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
2.951	124274	100.000	1.230	4320

Fig 4: Assay of tablet injection 2

Table 03: summary of injection 2

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

Limit of detection(LOD) -Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels. The values of Sy and slope were obtained when creating calibration curve in MS Excel using “SLOPE” and STEYX functions.

LOD = 3.3(Sy /S)

$$= \frac{3.3 \times 2027.6}{11762} = 0.57 \text{ µg /ml}$$

Limit of quantitation (LOQ) -Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels.

LOQ = 10(Sy /S)

$$= \frac{10 \times 2027.6}{11762} = 1.72 \text{ µg /ml}$$

The LOD and LOQ of Bisoprolol Fumarate were estimated as 0.57 µg/ml and 1.72 µg/ml respectively. The values indicated that the method was susceptible to quantify and detect the drug.

Precision:

Three QC standards were selected as LQC, MQC and NQC on the basis of calibration range.

LQC was concentration slightly more than lowest concentration of linearity. Lowest concentration in HPLC linearity study was 4µg/ml and next to it was 8 µg/ml, consequently, 18 µg/ml was selected LQC.

MQC was concentration near to middle concentration, slightly more or less and it was decided as 8µg/ml.

NQC was concentration near to the highest concentration but less than highest concentration. It was selected as 18µg/ml across the range.

Inter-day Precision

Area %RSD Calculations-

Conc	Area Day 1	Area Day 2	Area Day 3	Mean Area	% RSD
4µg/ml	48826	47892	47029	47915	1.8%
8µg/ml	94713	94152	94772	94545	0.36%
18µg/ml	208041	208761	207258	208020	0.36%

Table 04: Summary of Inter-day Precision of Area % RSD

Inter-day Precision

Retention Time %RSD Calculations-

Concentration	RT- Day 1	RT-Day 2	RT-Day 3	RT-Mean	% RSD
4µg/ml	2.944	2.944	2.942	2.943	0.04%
8µg/ml	2.942	2.947	2.945	2.944	0.08%
18µg/ml	2.948	2.945	2.946	2.946	0.05%

Table 05: Summary of Inter-day Precision of RT % RSD

Intra Day Precision:

Area % RSD Calculation :

Conc	Area Set I (morning)	Area Set II (Afternoon)	Area Set III (Evening)	Area-Mean	% RSD
4µg/ml	47974	47679	48266	47973	0.61%
8µg/ml	94637	94780	94159	94528	0.35%
18µg/ml	209338	207142	206767	207749	0.67%

Table 06: Summary of Intra-day Precision of Area % RSD

Intraday Precision

RT %RSD Calculations-

Conc	RT Set I (Morning)	RT Set II (Afternoon)	RT Set III (Evening)	RT-Mean	% RSD
4µg/ml	2.947	2.942	2.948	2.947	0.11%
8µg/ml	2.944	2.943	2.947	2.944	0.07%
18µg/ml	2.946	2.945	2.945	2.945	0.02%

Table 07: Summary of Intra-day Precision of RT % RSD

Accuracy:

Accuracy was conducted by analyzing 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. For all three levels, percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of % for Bisoprolol Fumarate.

Refer calibration curve for ‘ y ‘ value.

Calculations-

$$x = \frac{m}{y}$$

$$\% \text{ Recovery} = \frac{x}{T} * 100$$

Level	Bisoprolol Fumarate Stock solution taken	Tablet Stock Solution taken	Diluted with Mobile phase	Bisoprolol Fumarate (in µg/ml)
I (50%)	0.5 ml	1.0 ml	100 ml	15
II (100%)	1.0 ml	1.0 ml	100 ml	20
III (150%)	1.5 ml	1.0 ml	100 ml	25

Table 08: Solutions prepared by using stock solution

% Recovery of Bisoprolol Fumarate :

Theoretical Conc. (t)	Area obtained (m)	Slope (y)	Observed conc. in µg/ml (x)	% recovery
15 µg/ml	174590	11762	14.844	98.9%
20 µg/ml	228529	11762	19.429	97.1%
25 µg/ml	292504	11762	24.868	99.5%

Table 09: Summary of % Recovery

Discussion:

All the results found within the limit. The correlation coefficient (r2) was found to be not less than 0.99. %RSD for precision was found to be not more than 2. The mean recovery at all three levels was found to be not less than 98% and not more than 102%. The assay was found to be not less than 98% and not more than 102%.

Conclusion:

A new RP-HPLC method was developed for the simultaneous estimation of Bisoprolol Fumarate in their bulk I and solid dosage form i.e., tablet was developed and validate. The method reported in the present work has been effectively and efficiently used to analyze Bisoprolol Fumarate without any interference from the other excipients. Hence, it can be concluded that the new, simple, linear, precise, accurate, suitable and specific analytical method is developed and validated and can be used for routine analysis of its formulation.

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