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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF BISOPROLOL FUMARATE TABLETS

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ABSTRACT

A isocratic RP- HPLC method was developed for the determination of Bisoprolol fumarate in pharmaceutical dosage forms. The chromatographic separation was carried out on prontosil, chromo bond, $C_{18,}$ (250X4.6) mm, 5µ column and buffer (pH 5.6) and acetonitrile were mixed in the ratio of 750:250 was used as mobile phase at the flow rate of 1ml/min with PDA detection at 226nm. The retention time of Bisoprolol fumarate was found to be 9.15min. The developed HPLC method was validated by determining its sensitivity, selectivity, linearity, accuracy and precision, ruggededness and robustness. The assay method was found to be linear from 25 to 100 µg/ml. The accuracy of the method was assessed by percentage recovery studies at six different levels at 50%, 80%, 100%, 150%, 200% and 300% of its working concentration. The percentage recovery of the drug in the developed method was found to be in the ranges of from 97 to 103% that indicates the good accuracy of the method. This developed method can be used for the routine analysis for the estimation of Bisoprolol fumarate in bulk and Pharmaceutical formulations.

KEY WORDS

Bisoprolol fumarate and RP-HPLC Method.

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INTRODUCTION[1-2]

Bisoprolol fumarate is chemically: (*RS*)-1-[4-[[2-(1-Methylethoxy) ethoxy] methyl] phenoxy] -3-[(1 methyl ethyl) amino] propan-2-ol fumarate. It is a Beta-adreno receptor antagonist and used as an Anti - Hypertensive Drug. It is official in BP, USP Bisoprolol fumarate alone (or) in combined

formulation with other drugs is reported to be estimated by HPLC and UV/VIS Spectrophotometric methods. Literature review revealed that no HPLC method has been reported for the estimation of Bisoprolol fumarate in Pharmaceutical formulations individually. The present work describes a new, simple, rapid, accurate and precise RP-HPLC method developed and validated for the estimation of Bisoprolol fumarate. The structure of Bisoprolol fumarate is shown in Figure No.1.

MATERIALS AND METHOD

Chromatographic separation was carried out on Shimadzu Prominence liquid chromatographic system equipped with quaternary pump, PDA detector and auto injector. LC solution software (Version 1.23) was used for the entire processing and data collection. All chemicals used were analytical grade and the solvents which are used in the mobile phase were HPLC grade. Reference standard and tablet formulation of Bisoprolol fumarate were obtained from formulation and development department of Genovo development services limited (Bangalore) for analysis.

Instrument specifications

HPLC : SHIMADZU 1200 series
Pump : LC-20AD
Auto sampler : G1313A and G1329A
Detector : SPD-M20A
Software : LC Solutions
Degasser : G1379A and G1322A
Thermo stated column compartment : CTO-20A
Diode array detector: SPD-M20A

Preparation of standard solution

Weighed accurately about 50 mg of Bisoprolol fumarate Working Standard and transferred into a 50 mL volumetric flask, then added 30 mL of diluent(mobile phase), sonicated to dissolve then made up the volume with diluent, and mixed well. Transferred 5mL of the solution to 100 mL volumetric flask and made up to the mark with diluent[3-4].

Preparation of sample solution

Weighed accurately and taken 10 whole tablets and transferred the 10 whole tablets, (equivalent to about 12.5 mg of Bisoprolol fumarate), into a 250 mL volumetric flask, added about 170 mL of diluent(M.P), and sonicated for 20 minutes with occasional shaking, made up with up to the mark with diluent, and mix well. Centrifuge the test solution at 3500 RPM for 10 mins and use supernatant solution or filter the test solution through using 0.45µm PVDF (or) Nylon 66 membrane filter. Reject first few mL of the filtrate.

Method development and validation[5-10]

The RP HPLC procedure was optimized with a view to develop an effective method for the estimation of Bisoprolol fumarate in tablet dosage forms. Preliminary tests were performed in order to select the adequate and optimum chromatographic condition. A Prontosil, chromo bond, $C_{18,}$ (250X4.6) mm, 5µ column(temp 35°C) was used as a stationary phase and the separation was achieved by using mobile phase consisting of Buffer and Acetonitrile were mixed in the ratio of 750:250 v/v in isocratic mode at the flow rate of 1 ml/min with PDA detection at 226 nm. Chromatogram of standard solution containing Bisoprolol fumarate is shown in Figure No.2. The developed HPLC method for the estimation of Bisoprolol fumarate was validated as per the ICH guideline in terms of specificity, linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification.

Specificity

The specificity of the method was determined by spiking the solution of placebo with the working standard solution containing Bisoprolol fumarate and this solution was analyzed as per the method described. The recorded chromatogram was compared with chromatogram of standard solution containing Bisoprolol fumarate to check the interference of the placebo with the response produced by Bisoprolol fumarate.

System suitability

The system suitability of the method was determined by five replicate analysis of the standard solution containing Bisoprolol fumarate to check the reproducibility of the chromatographic system. In this method the reproducibility of peak area (RSD),

retention time, theoretical plate and tailing factor of the peaks of Bisoprolol fumarate were checked.

Linearity

The linearity of the method was assessed by analyzing the standard solution containing Bisoprolol fumarate at 6 different levels from 25 µg/ml to 100 µg/ml of its working concentration. The calibration curve of peak area (vs) concentration was plotted and correlation coefficient and regression line equation was determined. The calibration curve of Bisoprolol fumarate is shown in Figure No.3.

Accuracy

Accuracy of the method was assessed by analyzing the solutions containing Bisoprolol fumarate at six different levels (50%, 80%, 100%, 150%, 200% and 300%) of its working concentration. Standard solutions were spiked with placebo and the percentage recovery of the drugs from the placebo was calculated.

Precision

(i) Method Precision (Repeatability)

The precision of test method is determined by performing assay of six samples, prepared from Bisoprolol fumarate Tablets 1.25 mg as per the test procedures. Relative Standard Deviation and Confidence limit of Assay results were calculated.

(ii) Intermediate Precision

To determine the intermediate precision of assay method, analyst to analyst variation study was conducted by two different analysts, by calculating the assay of six samples, prepared from Bisoprolol fumarate Tablets 1.25 mg as per the test procedures. Relative Standard Deviation and Confidence Limit of Assay results were calculated.

Ruggedness and robustness

The ruggedness of the method was ascertained by carrying out the assay of the sample on different instrument by different analyst using different columns. Robustness of the method was determined by analyzing the sample by deliberately changed chromatographic conditions such as change in mobile phase organic composition (±10%), flow rate (0.1mL/min), column temperature (±5°C) and pH of the bufffer (±0.2).

LOD and LOQ

The limit of detection and limit of quantification of Bisoprolol fumarate were calculated by using standard deviation of the responses and the slope of the calibration curve of Bisoprolol fumarate. LOD and LOQ were estimated by using the following formula,

LOD = (3.3Xσ) / S

LOQ = (10 X σ) / S

Where,

σ is the standard deviation of the response

S is the slope of the calibration curve.

Analysis of Bisoprolol fumarate in Tablet formulation

For the assay of Bisoprolol fumarate in tablet formulations, twenty tablets were weighed and the average weight of the tablets was calculated. The weighed tablets were crushed in to fine powder. A quantity of powder equivalent to12.5mg of Bisoprolol fumarate was transferred in to 250ml volumetric flask 170 mL of diluent (M.P) was added. The content of the flask was sonicated for 20 minutes and the volume was then made up to 250ml with diluent. The test solution is centrifuged at 3500 RPM for 10mins, supernatant solution is filtered through using 0.45µm PVDF (or) Nylon 66 membrane filter. From the resulting solution 10µl was injected in to the column and response was recorded under the same chromatographic conditions. Six such samples were prepared and analyzed in the same manner. The amount of Bisoprolol fumarate present in the sample was determined by comparing the mean peak area of sample with that of standard.

RESULTS AND DISCUSSION

A simple, accurate and precise RP HPLC method was developed for the simultaneous estimation of Bisoprolol fumarate in tablet dosage form. Specificity of the method was tested by comparing the response of blank, standard and placebo mixed sample solution. No interference of placebo was detected at the retention time of Bisoprolol fumarate. The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. The results of the system suitability test

were summarized in Table No.1. Linearity of the method was evaluated at 6 different concentration levels from 25-100 µg/ml for Bisoprolol fumarate. The drug was found to give linear detector response in the concentration under study with correlation coefficient of 0.999917 (Table No.2). Accuracy of the method was determined by recovery test. The percentage recovery was found to be in the range of 97% -103 % (Table No.3). All results indicate that the method is highly accurate. This method was validated for its method and intermediate precision. The results obtained were within the acceptable limit is summarized in Table No.4 and 5) the ruggedness and robustness of the method were determined and the % RSD of the results were found to be less than 2.0%, which demonstrate that the developed method is rugged and robust. The result for ruggedness is summarized in Table No.6 and 7. The result for robustness is summarized in Table No.8 to 11. All the results of validation parameters are summarized in the Table No.12. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures.

Table No.1: Results of system suitability parameters

S.No	System Suitability Parameters	Observed value	Acceptance criteria
1	The Tailing factor for Bisoprolol peak from first injection of standard solution.	1.3	NMT 2.0
2	The Relative Standard Deviation for Bisoprolol peak response from five replicate injections of standard solution.	0.1	NMT 2.0%
3	Theoretical Plate count for Bisoprolol peak from first injection of standard solution.	12328	NLT 2000

Table No.2: Linearity data

S.No	Sample No	Bisoprolol fumarate	
		Concentration in µg/ml	Response
1	01	25.0843	7022706
2	02	40.1348	11273032
3	03	50.1685	13914537
4	04	60.2022	16870962
5	05	75.2528	21032528
6	06	100.3370	27742810

Table No.3: Results of recovery studies of marketed formulation (Bisoprolol fumarate)

S.No	Sample No	Spike Level	'mg' added	'mg' found	% Recovery	Mean % Recovery
1	1	50%	50.610	50.570	99.925	100.3
2	2	50%	50.550	51.060	101.013	
3	3	50%	50.710	50.620	99.815	
4	4	80%	80.300	80.730	100.536	100.5
5	5	80%	80.700	80.910	100.260	
6	6	80%	80.100	80.660	100.693	
7	7	100%	100.800	101.740	100.930	101.4
8	8	100%	100.300	102.060	101.752	
9	9	100%	100.500	101.910	101.407	
10	10	150%	150.800	151.270	100.310	100.9
11	11	150%	150.200	151.800	101.063	
12	12	150%	150.500	152.610	101.400	
13	13	200%	200.400	201.040	100.317	100.2
14	14	200%	200.100	201.130	100.516	
15	15	200%	200.600	200.410	99.904	
16	16	300%	299.900	301.390	100.496	100.6
17	17	300%	300.200	302.490	100.764	
18	18	300%	300.800	302.080	100.425	

Table No.4: Method of Precision (Repeatability)

S.No	Sample No	% Assay for 1.25 mg
1	01	99.1
2	02	97.6
3	03	99.0
4	04	98.4
5	05	97.4
6	06	99.4
7	Average	98.5
8	Std Dev	0.830
9	% RSD	0.84
10	Confidence limit	±0.7

Table No.5: Results of Intermediate precision

S.No	Sample No	Analyst-1	Analyst-2
1	01	99.1	98.3
2	02	97.6	98.1
3	03	99.0	98.0
4	04	98.4	97.7
5	05	97.4	98.2
6	06	99.4	98.4
7	Average	98.5	98.1
8	Std Dev	0.830	0.248
9	% RSD	0.84	0.25
10	Confidence limit	±0.7	±0.2

Table No.6: Results of System to System Variability (Ruggedness)

S.No	Sample No	System-1	System -2
1	01	99.1	98.3
2	02	97.6	98.1
3	03	99.0	98.0
4	04	98.4	97.7
5	05	97.4	98.2
6	06	99.4	98.4
7	Average	98.5	98.1
8	Std Dev	0.830	0.248
9	% RSD	0.8	0.3
10	Confidence limit	±0.7	±0.2

Table No.7: Results of Column to Column Variability (Ruggedness)

S.No	Sample No	Column -1	Column -2
1	01	99.1	98.3
2	02	97.6	98.1
3	03	99.0	98.0
4	04	98.4	97.7
5	05	97.4	98.2
6	06	99.4	98.4
7	Average	98.5	98.1
8	Std Dev	0.830	0.248
9	% RSD	0.8	0.3
10	Confidence limit	±0.7	±0.2

Table No.8: Robustness results (Effect of Variation in Mobile Phase Composition)

S.No	Organic Variation	% Assay of Bisoprolol Fumarate					
		90% Acetonitrile		100% Acetonitrile		110% Acetonitrile	
		Test-1	Test-2	Test-1	Test-2	Test-1	Test-2
1	1.25 mg Tablets	98.6	97.8	98.4	98.3	98.0	98.1

Table No.9: Effect of Variation in Flow Rate (Robustness)

S.No	Flow Rate Variation	% Assay of Bisoprolol Fumarate					
		0.9 ml/min		1.0 ml/min		1.1 ml/min	
		Test-1	Test-2	Test-1	Test-2	Test-1	Test-2
1	1.25 mg Tablets	97.8	99.1	98.0	99.5	98.1	100.3

Table No.10: Effect of Variation in Column Temperature (Robustness)

S.No	Oven Temperature Variation	% Assay of Bisoprolol Fumarate					
		30°C		35°C		40°C	
		Test-1	Test-2	Test-1	Test-2	Test-1	Test-2
1	1.25 mg Tablets	97.7	100.6	98.0	99.5	98.1	99.5

Table No.11: Effect of Variation of Buffer pH in Mobile Phase (Robustness)

S.No	Buffer pH Variation	% Assay of Bisoprolol Fumarate					
		Buffer pH-5.4		Buffer pH-5.6		Buffer pH-5.8	
		Test-1	Test-2	Test-1	Test-2	Test-1	Test-2
1	1.25 mg Tablets	99.1	98.2	98.4	98.3	99.2	98.6

Table No.12: Results of Validation of the developed HPLC Method

S.No	Parameters*	Bisoprolol Fumarate
1	Linearity (µg/ml)	25-100
2	Correlation coefficient	0.999917
3	% Recovery	97% to 103%
4	System precision(% RSD)	0.16
5	Method precision (% RSD)	0.84
6	Intermediate precision (% RSD)	0.25
7	Robustness (%RSD)	NMT 2
8	Ruggedness (%RSD)	NMT 2

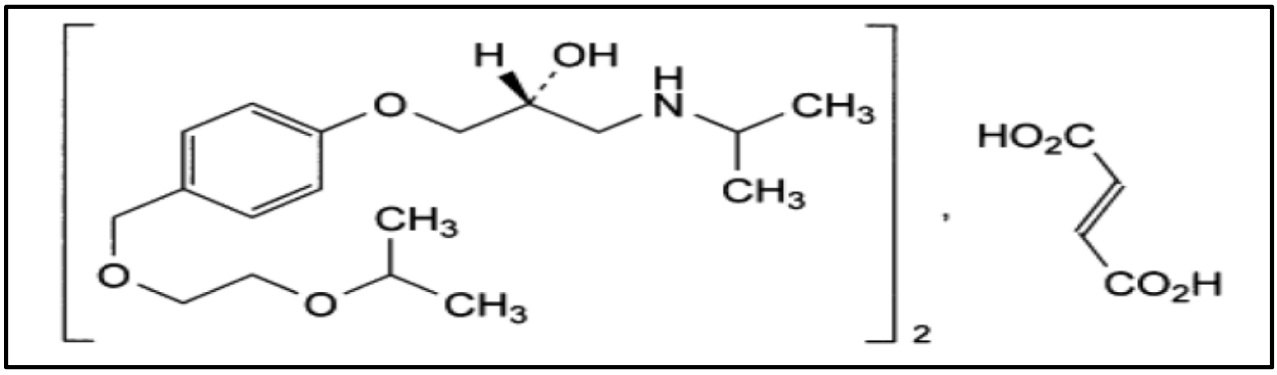

Figure No.1: Bisoprolol fumarate

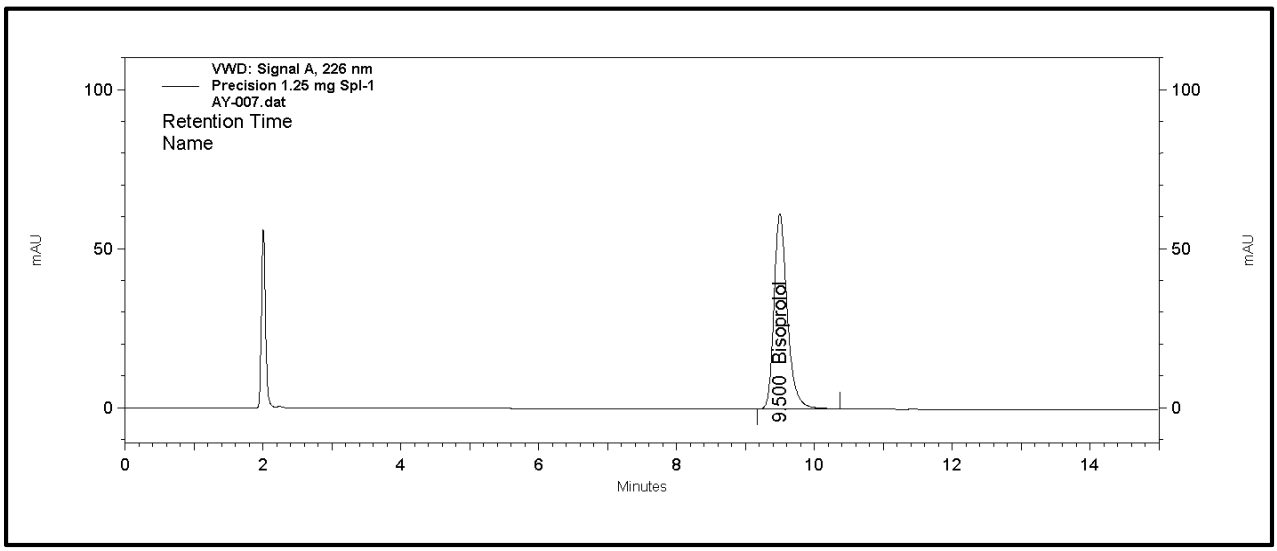

Figure No.2: Typical HPLC Chromatogram of Bisoprolol fumarate standard

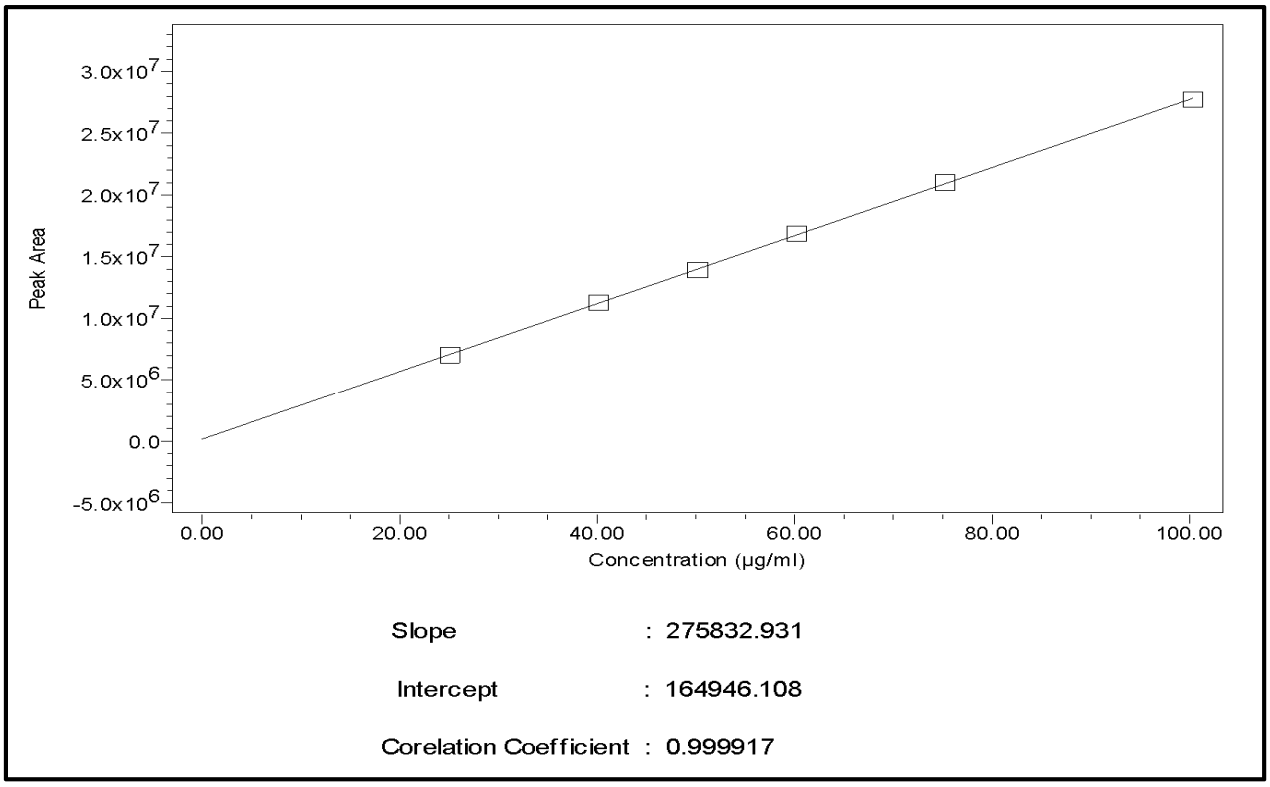

Figure No.3: Calibration curve of Bisoprolol Fumarate

CONCLUSION

The developed RP HPLC method for the simultaneous estimation of Bisoprolol Fumarate offers simplicity, selectivity, precision and accuracy. All the results of validation parameters are summarized in the Table No.3. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures. The method gives good resolution between the compounds with a short analysis time. So the developed method can be used for the routine analysis of Bisoprolol Fumarate in bulk and Pharmaceutical formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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