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Development and Validation of RP-HPLC Method for the Assay of Bisoprolol in Pure and Formulations

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ABSTRACT

A new RP-HPLC method development and validation of bisoprolol in pure and dosage forms was described. A Hypersil ODS (Make: Thermo; 150 mmx4.6 mm I.D; particle size 5µm) column was used for the present analysis of bisoprolol at ambient temperature. The mobile phase consisting of a mixture of buffer and acetonitrile in the ratio of 700:300 v/v was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20 µL. The photodiode array detector was set at a wavelength of 208 nm for the detection and chromatographic runtime was 9 minutes. The developed RP-HPLC method was validated as per ICH standards.

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INTRODUCTION

Bisoprolol, (±)-1-[4-[[2-(1-methyl ethoxy) ethoxy] methyl] phenoxyl-3-[(1-methyl ethyl) amino]-2-propanol (*E*)-2-utenedloate (2:1) (salt) is a cardio selective beta-blocker used in the treatment of hypertension and angina pectoris [1-3].

Literature survey revealed that few analytical methods are available for the estimations of Bisoprolol Fumarate in dosage forms [4-7] and in human plasma [8-10] that suffered from long retention times and use of expensive chemicals. Hence, in the present paper the author aimed to develop a simple, feasible, effective and economic validated RP-HPLC for quantification of bisoprolol in pharmaceutical formulation.

MATERIALS AND METHODS

Experimental

a. Chemicals and Reagents: Milli-Q Water, Methanol (HPLC Grade), Orthophosphoric acid (GR Grade), Potassium dihydrogen phosphate monohydrate (GR Grade) was obtained from Qualigens Ltd., Mumbai. All other chemicals of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware. Pharmaceutical grade bisoprolol were received as gift sample from Intas Pharmaceuticals Ltd, Ahmedabad, India. Marketed formulation, BISELECT Tablet (Intas Pharmaceuticals Ltd, Ahmedabad, India) containing 5.0 mg of bisoprolol was procured from the local pharmacy.

b. Instrumentation: The analysis of the drug was carried out on a waters LC system equipped with 2695 pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Hypersil BDS [(Make: Thermo); 150 mm X 4.6 mm I.D; particle size 5 µm)] was used. The output of signal was monitored and integrated using waters Empower 2 software.

c. Buffer Preparation: Dissolve 2.72 g of Potassium dihydrogen Phosphate and 0.1 % Tetra ethyl amine in 1000 mL of Milli-Q Water, adjust pH to 3.6 with dilute ortho-phosphoric acid and Filter the solution through 0.45 µm membrane filter.

d. Mobile Phase Preparation: Prepare a filtered and degassed mixture of buffer and acetonitrile in the ratio of 700:300 v/v respectively.

e. Diluent Preparation: Mobile Phase is used as diluent.

f. Standard Preparation: Accurately weigh and transfer about 100 mg of bisoprolol into a 100 mL volumetric flask, add 30 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent. From this working standard solutions were prepared in the concentration range of 5.0-17.5 µg/ml for bisoprolol by diluting suitable aliquots of the above stock solution with the same diluent into a series 25 mL volumetric flask respectively. Twenty micro liters of the test solution was injected and chromatogram was recorded for the same and the amounts of the drugs were calculated.

g. Sample Preparation: Weigh and finely powder not fewer than 10 Tablets (BISELECT Tablet, Intas Pharmaceuticals Ltd, Ahmedabad, India containing 5.0 mg of bisoprolol). Accurately weigh and transfer equivalent to 100 mg of bisoprolol into a 100 mL volumetric flask, add 70 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with the same diluent and mixed well. Filter the solution through 0.45 µm membrane filter. Later, transfer aliquots of the above filtrate into a series of 25 mL volumetric flasks and dilute to volume with diluent to obtain concentrations that obey within the standard limits. Twenty micro liters of the test solution was injected and chromatogram was recorded for the same and the amounts of the drugs were calculated.

RESULTS AND DISCUSSION

i. Method Development: Spectroscopic analysis of bisoprolol compound showed maximum UV absorbance (λ_{max}) at 208 nm, respectively. Therefore, the chromatographic detection was performed at 208 nm using a photo diode array detector as this drug exhibits good response at this λ_{max}. In developing a suitable LC method for bisoprolol assay different mobile phases were employed to achieve the best separation and resolution. Mobile phase consisting of the mixture of buffer and acetonitrile in the ratio of 500:500 v/v was used initially and it was observed that two drugs were eluted exhibiting a single split M shape peak.

In the next trial the mobile phase composition was changed slightly i.e., buffer and acetonitrile in the ratio of 600:400 v/v. The above said drugs were eluted with broad shaped peaks. When the mobile phase composition changed slightly to buffer and acetonitrile in the ratio of 700:300 v/v was injected into the column at flow rate of 1.0 mL/min. Bisoprolol were eluted with good peak shape with retention time of 3.146 minutes respectively. UV detection was performed at 208 nm. The chromatogram of bisoprolol standard using the proposed method is shown in (Fig.2). The system suitability results of the present proposed method are presented in Table 1.

Chromatographic Conditions: A Hypersil ODS (Make: Thermo; 150 mm X 4.6 mm I.D; particle size 5 µm) column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20 µL. The photodiode array detector was set at a wavelength of 208 nm for the detection and chromatographic runtime was 9 minutes. The typical chromatogram for the estimation of bisoprolol obtained by using the aforementioned mobile phase from 20 µL of the assay preparation is illustrated in Fig.2.

ii. Method Validation: The developed RP-HPLC assay method was validated in accordance with ICH guidelines using the following parameters.

a. System Suitability: System suitability parameters such as theoretical plates (USP) and asymmetry factor were evaluated injecting six replicated injections of standard solution of both bisoprolol. The results of the system suitability parameters were presented in Table 1.

b. Specificity

i. Blank and Placebo Interference: To establish the interference of blank and placebo with the proposed method the diluent and placebo was injected into the column under defined chromatographic conditions and the respective chromatograms were recorded. Chromatograms of blank and placebo solution showed no peaks at the retention time of bisoprolol peak indicating that the diluent and the placebo used in sample preparation did not interfered in the estimation of bisoprolol in market formulations.

c. Linearity of Detector Response: The standard curve was obtained in the concentration range of 5.00 - 17.5 µg/ml for bisoprolol. The slope, intercept and correlation coefficient [r^2] of

standard curve were calculated by linear regression analysis and given in Fig. 3 for bisoprolol demonstrating the linearity of the proposed method. From the data obtained in Table 2 the proposed RP-HPLC method was found to be linear within the proposed range.

d. Precision: The method precision study for six sample preparations in marketed sample showed % RSD of 0.058 and 0.12 for bisoprolol respectively revealing high precision of the proposed RP-HPLC method (Table 3**).**

e. Accuracy: The accuracy of the method was determined by the recovery studies were carried out in triplicate preparations on composite blend collected from 10 tablets of dosage form of bisoprolol and was analyzed as per the proposed method. The percentage recoveries found within the range of 98.84 % to 99.39 % for bisoprolol. From the data obtained in Table 4 the proposed RP-HPLC method was found to be accurate.

f. Robustness Studies: The robustness study of the developed assay method for bisoprolol was established for a few variance conditions [change in flow rate and change in organic phase in mobile phase]. The assay value of the test preparation solution was not affected and it was in accordance with that of actual. The results of system suitability parameters [Table 5] were also found satisfactory; hence, the analytical method would be concluded as robust.

g. Ruggedness Studies: Ruggedness studies for bisoprolol was determined between two different analysts, instruments and columns and the results of these studies were presented in Table 6. The values of percentage RSD for bisoprolol were below 2.0 % revealed the ruggedness of developed analytical method.

h. Analysis of Marketed Formulation: The proposed method was successfully applied to the determination of bisoprolol in their tablet dosage form (BISELECT). It is evident from these results [Table 7] that the proposed RP-HPLC method can be applicable to the analysis of bisoprolol in their commercial formulation with minimum sample preparation and satisfactory level of selectivity, accuracy and precision.

CONCLUSION

An RP-HPLC method for the estimation of bisoprolol in formulations was developed and validated as per ICH guidelines. The linearity of the proposed method was observed over a

concentration range of 5.0 - 17.5 µg/mL. All the results of other validation parameters indicated that the proposed method is rapid, accurate, selective, and reproducible. The mean recovery for bisoprolol from tablets was ranged 99.92 % making the advantage of this proposed method adopted for all formulations.

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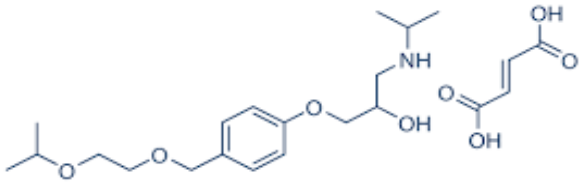
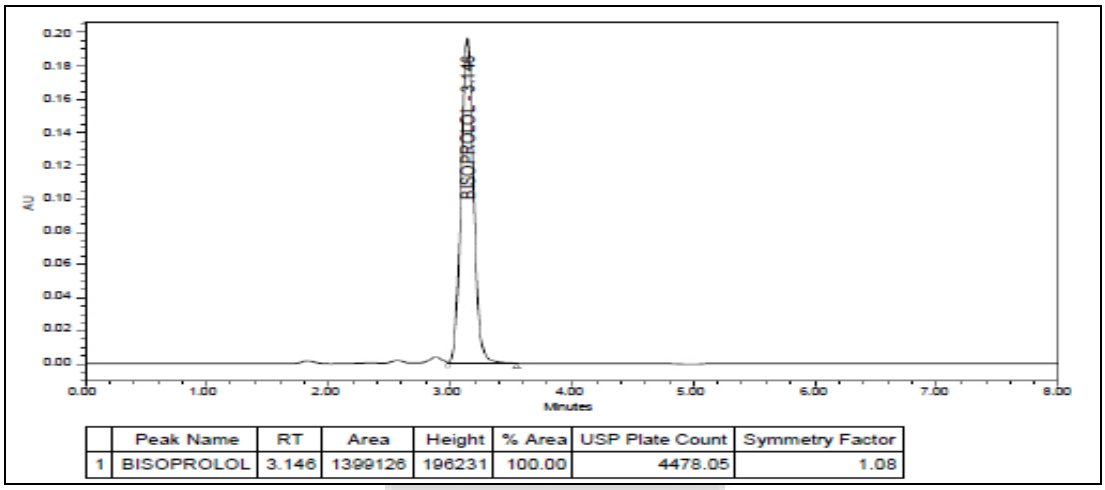


Figure 1. Molecular Structure of Bisoprolol



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor
1	BISOPROLOL	3.146	1399126	196231	100.00	4478.05	1.08

Figure 2. Validative Chromatogram of Bisoprolol

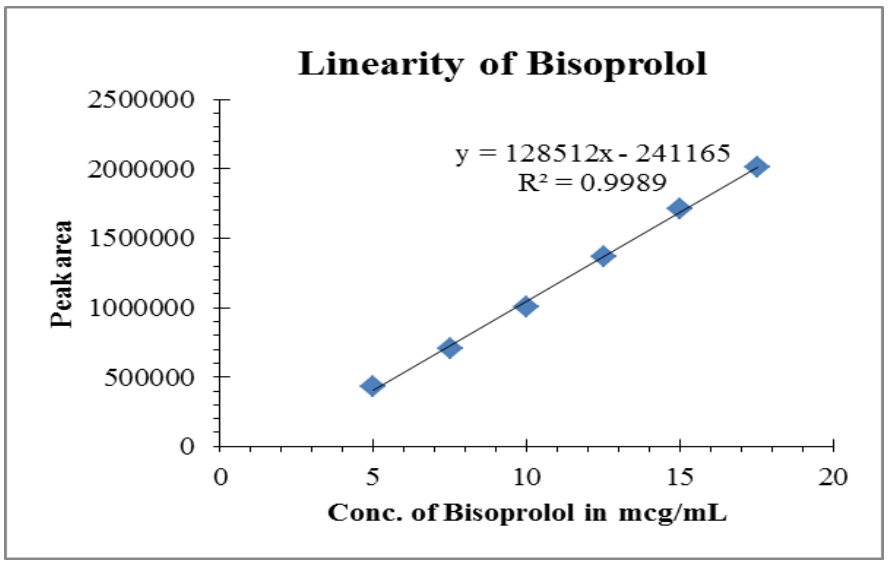


Figure 3. Linearity Plot of Bisoprolol

Table 1: System Suitability Parameters for Bisoprolol by Proposed Method

Name of the Compound	Retention Time	Theoretical Plate	USP Resolution
BISOPROLOL	3.146	4478.05	1.08

Table 2: Linearity Studies of Bisoprolol by the Proposed Method

Linearity Study For Bisoprolol		
% Level	**Conc. [µg/Ml]**	**Area**
25	5.0000	435093
50	7.5000	705385
75	10.0000	1002205
100	12.5000	1367301
125	15.0000	1708252
150	17.5000	2009307
Slope		128512
Intercept		-241165
RSQ(r2)		0.9989
LOD (µg/mL)		0.812
LOQ (µg/mL)		2.70

Table 3: Method Precision Studies for Bisoprolol by Proposed Method

Sr. No.	Name	Rt	Area
1	SOLUTION-1	3.151	1402132
2	SOLUTION-2	3.153	1402072
3	SOLUTION-3	3.15	1400323
4	SOLUTION-4	3.149	1397965
5	SOLUTION-5	3.149	1399055
6	SOLUTION-6	3.153	1399767
AVG*		3.151	1400219
STD DEV*		0.002	1657.9
% RSD*		0.058	0.12

*Average of six determinations

Table 4: Recovery Studies for Bisoprolol by Proposed Method

Sr. No.	BISOPROLOL		
% SPIKED LEVEL	**50%**	**100%**	**150%**
INJECTION 1	1058435	1399766	1754320
INJECTION 2	1053208	1400435	1751024
INJECTION 3	1058979	1406456	1753450
*AVERAGE	1056874	1402219	1752931
*AMOUNT RECOVERED (µg)	49.69	99.19784	148.26
*% RECOVERY	99.39	99.19	98.84

*Average of three determinations

Table 5: Robustness Studies of Bisoprolol

Robust Conditions		Bisoprolol	
		Peak Area	USP Tailing
Flow Rate	0.8mL/min.	1049878	1.12
	1.2mL/min.	1055567	1.09
Organic Composition	10% less	1067888	1.11
	Actual	1047899	1.10

Table 6: Ruggedness Data for Bisoprolol

Sr. No.	Name	RT	Area	RT	Area
1	Injection-1	3.151	1402132	3.152	1405477
2	Injection-2	3.153	1402072	3.155	1402154
3	Injection-3	3.15	1400323	3.154	1400323
4	Injection-4	3.149	1397965	3.151	1405458
5	Injection-5	3.149	1399055	3.157	1407643
6	Injection-6	3.153	1399767	3.157	1407098
Avg*		3.151	1400219	3.154333333	1404692.167
Std Dev*		0.002	1657.9	0.002503331	2871.578759
% RSD*		0.058	0.12	0.079	0.204

*Average of six determinations

Table 7: Results of HPLC Analysis of Formulations

Drug	Label Claim	*Quantity Found	% Assay
Bisoprolol	5.0	4.96	99.92

*Average of three determinations