

## DEVELOPMENT AND VALIDATION OF HPLC-DAD METHOD FOR THE DETERMINATION OF BISOPROLOL IN TABLET DOSAGE FORMS

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### ABSTRACT

**Objective:** A rapid, simple and sensitive RP-HPLC method was developed and validated for the determination of bisoprolol fumarate in bulk and pharmaceutical dosage form.

**Methods:** Chromatographic separation was achieved within 2.5 min on ACQUITY Arc System, Waters Symmetry C18 column (3.9 mm i.d. X 150 mm, 5 µm particle sizes) using a mobile phase consisted of acetonitrile: phosphate buffer (25:75 v/v) in an isocratic mode at a flow rate of 1.4 ml/min. The pH of the mobile phase was adjusted to 7.0 with orthophosphoric acid and UV detection was set at 226 nm.

**Results:** The retention time for bisoprolol fumarate was found to be 2.09 min. The proposed method was validated according to ICH guidelines with respect to linearity, specificity precision, accuracy and robustness. The limit of detection and limit of quantification are calculated and found to be 0.4825 and 1.4621 µg/ml; respectively.

**Conclusion:** The proposed method can help research studies, quality control and routine analysis with lesser resources available. The results of the assay of pharmaceutical formulation of the developed method are highly reliable and reproducible and is in good agreement with the label claim of the medicines.

**Keywords:** Bisoprolol, High-Performance Liquid Chromatography, Validation, ICH guidelines

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### INTRODUCTION

Nowadays in the pharmaceutical analysis is dominated by physico-chemical methods of analysis, the most reliable of which are methods of chromatographic analysis. Chromatographic methods allow to carry out qualitative and quantitative determination of AFI.

Bisoprolol is a synthetic, beta1-selective (cardioselective) adrenoceptor blocking agent without significant membrane stabilizing activity or intrinsic sympathomimetic activity in its therapeutic dosage range. The chemical name of bisoprolol fumarate is 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy] propan-2-ol (fig. 1). The two substituents present in the para position of the benzene ring might be the reason for its β1-adrenergic receptor selectivity. The most prominent effect of bisoprolol fumarate is the negative chronotropic effect, resulting in a reduction in resting and exercise heart rate. There is a fall in resting and exercise cardiac output with little observed change in stroke volume, and only a small increase in right atrial pressure, or pulmonary capillary wedge pressure at rest or during exercise [1].

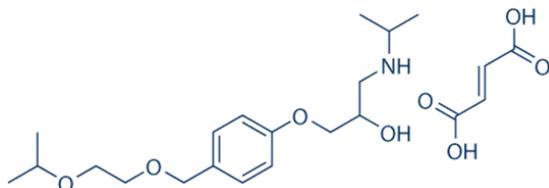


Fig. 1: Chemical structure of bisoprolol fumarate

A literature survey was conducted and several methods were reported for the determination of bisoprolol fumarate, such as spectrophotometry [2-5] and liquid chromatography [6-12].

However, no rapid and sensitive HPLC for the determination of bisoprolol in its tablet dosage form.

This novel proposed method contributes quick estimation, correct peak shape, precise, simple, and quick, use of smaller sample volumes and acetonitrile and phosphate buffer solution pH 7.0 as a mobile phase which is economical when compared with other existing methods [13].

The present work aimed to develop a simple, rapid, sensitive and specific method for the determination of bisoprolol fumarate in its tablet dosage form according to ICH guidelines Q2 (R1) [14].

### MATERIALS AND METHODS

#### Chemicals and reagents

Bisoprolol fumarate was obtained as a gift from Farmak pharmaceuticals (Kiev, Ukraine). HPLC grade acetonitrile, triethylamine, ammonium phosphate, orthophosphoric acid were obtained from Merck pharmaceuticals.

#### Instrumentation and chromatographic conditions

ACQUITY Arc System, Waters Symmetry C18 column (3.9 mm i.d. X 150 mm, 5 µm). Chromatographic separation was carried out at ambient temperature (22 °C-25 °C). The compound was separated isocratically with a mobile phase consisting of acetonitrile and phosphate buffer solution pH 7.0 (25/75, v/v) at a flow rate 1.4 ml/min with injection volume 10 µL. The effluent was monitored spectrophotometrically at wavelength 226 nm.

#### Preparation of mobile phase

To prepare phosphate buffer solution pH 7.0: 6.60g of ammonium phosphate dissolve in 980 ml of water, add 2.0 ml of triethylamine, fix the pH of the solution to the value (7.0±0.05) with phosphoric acid, and then add the volume of the solution with water P to 1000.0 ml and mix.

### Stock standard solutions

Primary stock solutions of bisoprolol fumarate was prepared daily separately by dissolving 25 mg of each in 250 ml volumetric flasks (0.1 mg/ml) in mobile phase. Limits of the range of application of the procedure were determined from 70% to 130% to the nominal concentration of bisoprolol fumarate (0.1 mg/ml) in the test solution.

### Procedures

The standard solutions were prepared by dilution of the stock standard solution with mobile phase. Triplicate 20.0  $\mu$ L injections were made for each concentration and chromatographed under the condition described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph and regression equation was computed.

### Analysis of dosage form

Accurately weighed twenty bisoprolol tablets and average weight was calculated. Accurately weighed a portion of tablet, powder equivalent to 10 mg of bisoprolol fumarate and transfer into a 100 ml volumetric flask to this 50 ml solvent (water-acetonitrile 80:20 v/v) was added and sonicated for 15 min. Solvent (water-acetonitrile 80:20 v/v) was adjusted up to the mark. The solution was filtered using 0.45  $\mu$ m nylon filter. The amount present in the tablet was calculated from plotted calibration graph or utilizing regression equation. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 10  $\mu$ L fixed sample loop of the injection port.

### Method validation

Once the chromatographic and the experimental conditions were established, the method was validated with respect to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ) according to ICH Q2 (R1) guidelines [15, 16].

### System suitability parameters

The chromatographic systems used for analysis must pass system suitability before going to start the experiment. At first HPLC system is stabilized for forty minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters such as tailing factor, theoretical plate count and retention time. The parameters such as tailing factor, % RSD and theoretical plates were studied.

### Linearity

A standard stock solution of the bisoprolol fumarate (0.1 mg/ml) was prepared with the mobile phase. To study the linearity range of drugs, serial dilutions were made from standard stock solution.

### Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from

placebo and degradation products. The specificity of the method was established by injecting blank, placebo and standard solution in triplicate and recording the chromatograms.

### Precision

The precision of the method was determined by repeatability (intraday) and intermediate precision (interday). Repeatability was determined by performing six repeated analysis of the same working solution of bisoprolol fumarate on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on different days and also by another analyst performing the analysis in the same laboratory (between-analysts).

### Accuracy

The accuracy of a method is defined as the closeness of a measured value to the true value. The recovery studies were carried out at 70-130 % of the target level in the tablet in triplicate each in the presence of placebo.

### Robustness

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered to be: variations in the flow rate, detection wavelength and mobile phase composition. There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing thus that the method is robust. The % RSD of bisoprolol fumarate should be not more than 2.0 %.

### LOD and LOQ

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

## RESULTS AND DISCUSSION

### Method development and optimization

The RP-HPLC method was developed to provide specific procedure for the rapid quality control analysis of bisoprolol fumarate. To find the appropriate HPLC conditions for separation of the examined drug, various reversed phase columns, isocratic and gradient mobile phase systems were tried, and successfully attempts were performed using a RP-C18 chromatographic column Symmetry C18 column (3.9 mm i.d. X 150 mm, 5  $\mu$ m) and mobile phase composed of acetonitrile: phosphate buffer solution pH 7.0 in the ratio of 25:75 v/v, at a flow rate of 1.4 ml/min with  $\lambda_{max}$  at 226 nm. Under the described HPLC parameters, the respective compound was clearly separated and their corresponding peaks were sharply developed at reasonable Rt 2.09 min as shown in (fig. 2). Previous studies show more Rt than 2.09 and it means that developed method is more rapid [6-13]. The optimum chromatographic conditions and system suitability parameters are tabulated in table 1.

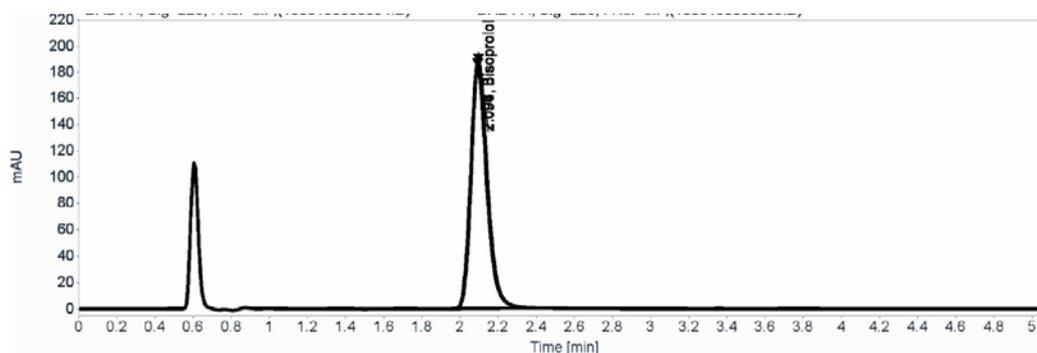


Fig. 2: Representative chromatogram of USP bisoprolol fumarate (0.1 mg/ml) using UV detection at 226 nm

Table 1: Optimized chromatographic conditions

Parameter	Chromatographic conditions
Instrument	ACQUITY Arc System
Column	Waters Symmetry C18 column (3.9 mm i.d. X 150 mm, 5 µm)
Mobile phase	Acetonitrile: phosphate buffer solution pH 7.0 (25/75, v/v)
Flow rate	1.4 ml/min
Detection wavelength	UV at 226 nm
Runtime	10 min
Column temperature	35 °C
Volume of injection loop	10 µl
Retention time	2.09 min

\*= number of 5 determinations.

### System suitability

According to U. S. Pharmacopeia (USP) states that system suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.

For HPLC method, it was used to verify that the column efficiency (N), selectivity factor (resolution) and reproducibility of the chromatographic system. Parameters including resolution (Rs), peak symmetry, capacity factor (K') and selectivity factor ( $\alpha$ ) were calculated, as shown in table 2. All the values are well within the limit.

Table 2: Analytical parameters for system suitability test of HPLC

Parameters	Reference	Bisoprolol fumarate
Retention time (min)	-----	2.09
Resolution (R)	R>2	-----
Selectivity factor ( $\alpha$ )	>1	
K' (Column capacity)	0.1-10 acceptable	
Symmetry	-----	
Tailing Factor (T)	$\leq 2$	1.12
N (column efficiency)	Increases with efficiency of the separation	12215
Height equivalent theoretical plates (HETP)	The smaller the value, the higher the column efficiency =L/N	0.012

### Method validation

#### Linearity

Calibration curve representing the relation between the concentrations of drugs versus the peak area were constructed. In triplicate run from which the linear regression equation was calculated. The results of

chromatographic determination of nine model solutions of bisoprolol fumarate are present in table 3. The calibration plot of bisoprolol fumarate is presented in (fig. 3) and characteristics of the linear dependence of bisoprolol fumarate are listed in table 4.

The results show that a phenomenal relationship between peak area and concentration of the drug in the calibration curve.

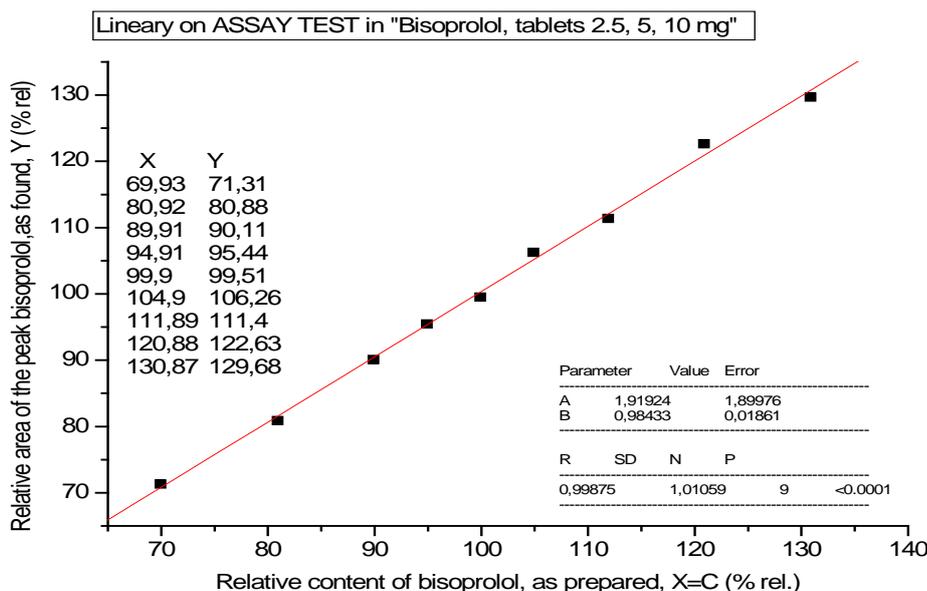


Fig. 3: The calibration graph of bisoprolol fumarate

Table 3: Chromatographic determination of model solutions of bisoprolol fumarate

Model solutions	Assigned API content, X <sub>i</sub> (%)	S <sub>i</sub> parallel measurements	Average S <sub>i</sub>	RSD <sub>i</sub> , %	Found content of API, Y <sub>i</sub> (%)
1	69.93	774.132 774.227 774.029 774.594 774.221	774.241	0.03	71.31
2	80.92	879.099 878.136 879.243 877.213 876.681	878.074	0.13	80.88
3	89.91	978.703 976.504 978.890 978.484 979.049	978.326	0.11	90.11
4	94.91	1036.698 1037.089 1033.310 1036.406 1037.456	1036.192	0.16	95.44
5	99.90	1080.720 1079.984 1080.441 1080.173 1080.298	1080.323	0.03	99.51
6	104.90	1153.316 1154.312 1153.254 1153.666 1153.455	1153.601	0.04	106.26
7	111.89	1209.429 1209.652 1209.619 1210.412 1208.281	1209.479	0.06	111.40
8	120.88	1332.491 1330.725 1331.603 1331.185 1330.739	1331.349	0.06	122.63
9	130.87	1407.595 1409.233 1407.299 1407.680 1407.949 1086.486 1085.791 1084.526 1085.396	1407.951	0.05	129.68

\*Average of triplicate injections

Table 4: Characteristics of the linear dependence of bisoprolol fumarate

The name of the quantity	Value	Criteria for the parameters of linear dependence (according to SPU 1.2, p. 85)
B	0.98433	
S <sub>B</sub>	0.01861	
A	1.91924	≤ 2.56
S <sub>A</sub>	1.89976	
S <sub>R</sub>	1.01059	
S <sub>R</sub> /B	1.02614	≤ 1.27
R	0.99875	≥ 0.997789

The results obtained were processed by the least squares method. The correlation coefficient of bisoprolol fumarate (r<sup>2</sup>) was noted as 0.99875 which states that the method was good linear to the concentration versus peak area responses. Results indicate high sensitivity of the proposed HPLC method.

#### Specificity

Commonly used tablet excipients did not interfere with the proposed method. The specificity results are tabulated in table 5. Furthermore, the well shaped peaks also indicate the specificity of the method.

Table 5: Specificity study

Name of the solution	Retention time (t <sub>R</sub> ) min
mobile phase	No peaks
placebo	No peaks
bisoprolol fumarate 0.5 mg/ml	2.09

**Accuracy and precision**

Intra-day and inter-day precision represented as % RSD values lower than 2% clearly assuring that this method was found to be

fairly precise and reproducible as shown in Tables 6,7. The standard addition method was performed at 70 %, 80 %, 90 %, 100 %, 110 %, 120 %, 130 % levels as illustrated in table 8. % RSD was lower than 2 % proving the high repeatability of the new method.

Table 6: Results of system precision

Sample	Concentration (µg/ml)	Peak area	Injection no.	RSD, %
Bisoprolol	0.1	1086.213	1	0.07
		1086.486	2	
		1085.791	3	
		1084.526	4	
		1085.396	5	

\*Each value is represented as a mean±SD of 4 observations (n=4), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria<2.0.

Table 7: Intra-day and Inter-day precision data of bisoprolol fumarate

Day	Intra-day precision		Inter-day precision	
	Mean	RSD %	Mean	RSD %
1	99.82	0.311	100.76	0.364
2	100.41	0.647	99.27	0.390
3	100.82	0.336	100.53	0.572

\*Each value is represented as a mean±SD of observations, SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria<2.0

Table 8: Evaluation of accuracy of the proposed HPLC method

Model solutions	The amount of bisoprolol fumarate, %		Found, % to predetermined, Z <sub>i</sub> = (Y <sub>i</sub> /X <sub>i</sub> )-100%
	Predetermined quantity, X <sub>i</sub> =(m <sub>i</sub> /m <sub>rs</sub> ) 100 %	Found quantity, Y <sub>i</sub> =(S <sub>i</sub> /S <sub>rs</sub> ) 100 %	
1	69.93	71.31	101.98
2	80.92	80.88	99.95
3	89.91	90.11	100.22
4	94.91	95.44	100.56
5	99.90	99.51	99.60
6	104.90	106.26	101.30
7	111.89	111.40	99.56
8	120.88	122.63	101.44
9	130.87	129.68	99.09
Average, Z, %			100.41
Standard deviation, S <sub>z</sub> , %			0.98
Confidence interval of convergence of results (actual)			1.82
$\Delta = t(95\%,8) \cdot S_z = 1.8595 \cdot S_z$ , %			
Critical value for the convergence of results			Performed
$\Delta \leq \max \Delta_{As} = 2.4\%$			(1.82<2.4)
Systematic error $\delta =  Z-100 $ , %			0.41
Criterion of significance of systematic error			Performed
$\delta \leq \max \delta$ , %			(0.41<0.77)
The general conclusion about the technique:			Correct

\*Average of triplicate injections

Regarding accuracy, a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution. The percentage recovery was calculated by comparing the area before and after addition of the standard drug. These data showed that the proposed method was both accurate and precise for the determination of bisoprolol fumarate in medicines. The high value of

recoveries obtained for bisoprolol indicates that the proposed method was found to be accurate.

**LOD and LOQ**

The LOD and LOQ values are presented in table 9.

Table 9: LOD and LOQ of the proposed HPLC method for bisoprolol fumarate

Limit of Detection (LOD)	0.4825 µg/ml
Limit of Quantitation (LOQ)	1.4621 µg/ml

The developed method found to be high sensitivity with LOD and LOQ of 0.4825 and 1.4621 µg/ml; respectively. The results of LOD and LOQ supported the sensitivity of the proposed method.

**Table 10: Robustness of the proposed HPLC method for bisoprolol fumarate**

Parameter	Optimized	Used	Retention time (min)	Remark
Flow rate ( $\pm 0.2$ ml/min)	1.4 ml/min	1.2 ml/min	2.11	Robust
		1.4 ml/min	2.09	Robust
		1.6 ml/min	2.09	Robust
Detection wavelength ( $\pm 5$ nm)	226 nm	221 nm	2.10	Robust
		226 nm	2.09	Robust
		231 nm	2.11	Robust
Mobile phase composition (acetonitrile: phosphate buffer solution pH 7.0) ( $\pm 0.5$ %)	25:75 v/v	30:70 v/v	2.12	Robust
		25:75 v/v	2.09	Robust
		20:80 v/v	2.11	Robust

It was evaluated by small deliberate changes in method parameters such as flow rate ( $\pm 0.2$  ml/min), detection wavelength ( $\pm 5$  nm) and mobile phase composition ( $\pm 0.5$  %). The % RSD values of robustness which is less than 2 % reveals that the proposed method is robust. The results of robustness indicate that even though the small changes in the conditions did not significantly effect on retention time of bisoprolol fumarate.

**Table 11: Results of analysis of bisoprolol fumarate**

S. No	Formulation	Labelled amount, mg/tablet	Amount found, mg/tablet	Mean % assay $\pm$ SD	% RSD
1	Bisoprolol tablets	5	4.98	99 $\pm$ 1	0.100

\*Average of 5 determinations; SD: standard deviation; RSD: relative standard deviation.

### Robustness

The results of robustness study results are shown in table 10.

### Tablet analysis

The developed, validated method was successfully applied for the determination of bisoprolol fumarate in their tablet dosage form. The assay result shows that the amount of the drug was in excellent agreement with the labelled value of the formulation as illustrated in table 11.

### CONCLUSION

In conclusion, a rapid, simple, accurate, sensitive and precise HPLC method was developed and validated for the estimation of bisoprolol fumarate in its tables dosage form. Statistical analysis for the above results obviously demonstrates that the method is fit for the estimation of bisoprolol fumarate in tablet forms without any interference. The proposed method can help research studies, quality control and routine analysis with lesser resources available. The results of the assay of pharmaceutical formulation of the developed method are highly reliable and reproducible and is in good agreement with the label claim of the drug.

### CONFLICT OF INTERESTS

Declared none

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