

# A HPLC Method for the Determination of Bisoprolol in Tablets and its Application to a Bioequivalence Study

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*A fast, robust RP-HPLC method was developed for determination of bisoprolol fumarate in tablets. The mobile phase was a mixture of methanol:acetonitrile:45mM potassium dihydrogen phosphate buffer (30:25:45) at pH 3.0 and 0.3mL/min. The stationary phase used was a SB-C<sub>18</sub> Nucleosil column (125×4mm). The UV detection was performed at 225nm. The method was validated as far as linearity, limit of detection, limit of quantification, precision, accuracy, recovery and system suitability. The retention time for bisoprolol was 2.32 min. The calibration graph was linear in the concentration range 0.3-10 µg/mL. The assay proved to be sensitive, specific and reproducible. The method was applied for the determination of bisoprolol in tablets and in a bioequivalence study.*

**Keywords:** bisoprolol, HPLC method validation, bioequivalence study.

Bisoprolol fumarate is a synthetic beta 1 – selective blocking agent. It is used in the treatment of cardiovascular diseases such as high blood pressure (hypertension), reduced blood flow to the heart (cardiac ischemia) and congestive heart failure. It is also used as preventive treatment before and as primary treatment after heart attacks decreasing the chances of recurrence [1]. Bisoprolol has a higher degree of beta 1 - selectivity compared to other beta 1 - selective beta – blockers such as atenolol, metoprolol and betaxolol [2-4]. The beta 1 – selectivity of bisoprolol fumarate has been demonstrated in both animal and human studies.

The chemical name for bisoprolol fumarate is (±)-1-[4-[[2-(1-methylethoxy) ethoxy] methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol(E)-2-butenedioate [5]. Its empirical formula is (C<sub>18</sub>H<sub>31</sub>NO<sub>4</sub>)<sub>2</sub>·C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> and its structure is presented in figure 1:

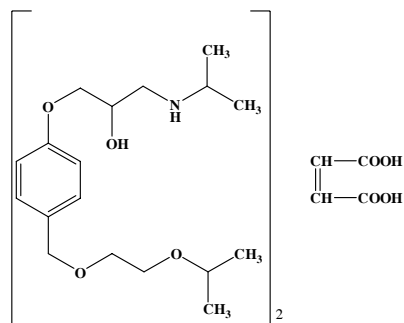


Fig. 1. Structural formula of bisoprolol fumarate

Various methods for bisoprolol determination are reported in literature. There are UV-Vis [6, 7], HPLC [8 – 10], HPTLC [11, 12] methods for the determination of bisoprolol from tablets and biological fluids.

The aim of this study was to develop a fast, efficient, accurate, precise and robust RP-HPLC method for the separation and quantitative determination of bisoprolol from tablets.

## Experimental part

### Instruments

An Agilent Technologies 1100 High Performance Liquid Chromatograph with Diode Array Detector, a Kern 770 analytical balance and a Cole Parmer ultrasonic bath were used [13-15].

The chromatographic separation of bisoprolol was achieved using isocratic elution and a reverse phase column (Zorbax SB-C18 100×3mm, 3.5 µm). The chromatographic assay was performed at 25°C using a 20 µL sample loop.

### Reagents

The solvents and other materials were HPLC grade provided by Merck's Chemical Co and Fluka. The bisoprolol reference substance was supplied from Unichem Laboratories LTD, India. Pharmaceutical formulations of 5 and 10mg bisoprolol tablets were acquired from local pharmacies.

### Mobile Phase

The mobile phase consisted from a 30:25:45 mixture of methanol, acetonitrile and 45mM potassium dihydrogen pH 3.0 phosphate buffer and it was pumped into the system with a flow rate of 0.30mL/min.

### Preparation of solutions

100 µg/mL stock standard solution of bisoprolol was prepared using mobile phase and then stored at room temperature. The stock standard solution was diluted with mobile phase to appropriate working solution (10 µg/mL).

A phosphate buffer (45mM) was prepared by dissolving 6.12g of monopotassium phosphate in 900mL water. The solution was brought to 1000mL with distilled water and the pH was adjusted to 3.0 with orthophosphoric acid.

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### Sample preparation

The developed method for the determination of bisoprolol was applied to a quality control study of pharmaceutical tablets from various manufacturers. Twenty bisoprolol fumarate tablets were crushed and a 5mg bisoprolol fumarate equivalent quantity of powder was dissolved in 25mL methanol using an ultrasonic bath and then it was filtered in order to separate the excipients. It was all brought to 100mL using mobile phase. Three successive samples were obtained.

### The bioequivalence study

The pharmaceutical monography for tablets provides a quantitative determination of the active substance released after tablet dissolution. The tablets analysed were Concor® (original product) and Bisotens® (generic product), both containing 10mg bisoprolol fumarate. The dissolution test was carried out on 6 tablets, using phosphate buffer as dissolution medium, according to United States Pharmacopeia 29 (USP) [5], at three different pH values (1.2, 4.5 and 6.8) using an Agilent Technologies 708 dissolution apparatus. The dissolution mediums were prepared according to European Pharmacopoeia 6.0 [13-15].

### Method validation

Validation of the method was carried out following the norms of The International Conference on Harmonization (ICH) [16] guidelines for selectivity, linearity, detection limit, quantification limit, system suitability, precision, accuracy, recovery, stability and robustness.

The linearity was investigated in the 0.10-15µg/mL concentration range and the calibration curve was obtained by plotting the peak area values against the bisoprolol fumarate concentration using linear regression.

The detection limit (LOD) and the quantification limit (LOQ) were calculated using the calibration curve statistical parameters in the following formulas (1):

$$LOD = \frac{3.3 \times SD}{S} \quad LOQ = \frac{10 \times SD}{S} \quad (1)$$

where S was the slope of the calibration curve and SD was the standard deviation of the intercept of from regression equation [16-19].

The precision and accuracy were assessed by determining the active compound concentration at three concentration levels in the same day and in three different days. The precision of the method was evaluated through standard deviation (SD) and covariance (CV) (%), respectively. The accuracy was evaluated through recovery.

The recovery of tablet extraction for three bisoprolol fumarate concentrations was determined. Known amounts of standard bisoprolol fumarate were added to tablets solutions. After the chromatographic analysis, the peak areas were compared to those obtained for standard solutions of bisoprolol fumarate with equal concentration.

To evaluate the robustness of the developed RP-HPLC method, some small deliberate variations in the optimized method parameters were made. The effects of modifying flow rate or column temperature on the peak area were studied.

A series of standards samples was prepared from fresh stock solutions in the mobile phase to evaluate the stability of samples at room temperature.

## Results and discussions

### Optimization of chromatographic conditions

Several parameters were examined for the optimization of HPLC analysis of the bisoprolol fumarate. The first step

was establishing the composition of the mobile phase and the retention time (fig. 2). According to the obtained results it was established that the best mobile phase was a mixture of acetonitrile, methanol and pH 3.0 phosphate buffer and the retention time of bisoprolol fumarate was 2.32min.

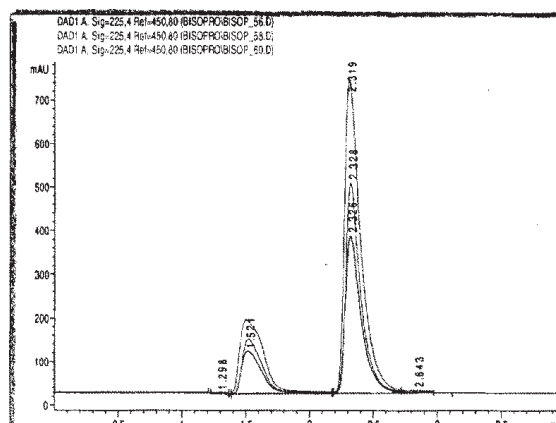


Fig. 2. Retention time

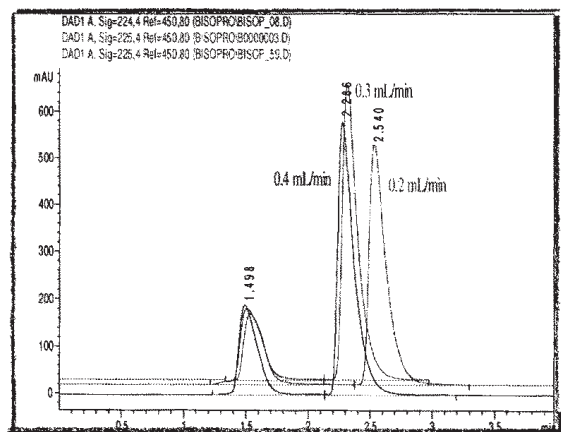


Fig.3. The flow rate study

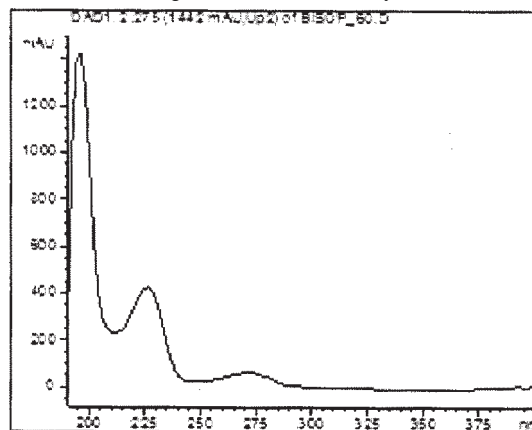
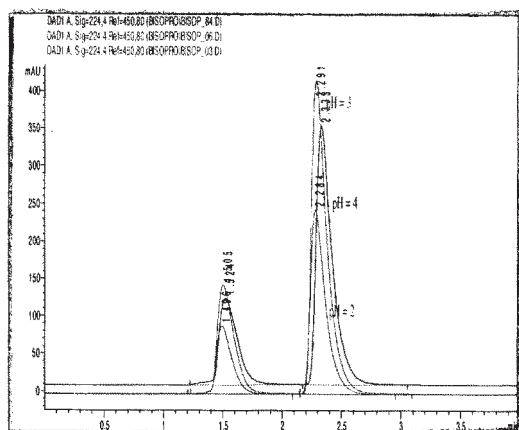


Fig.4. The absorption spectra of bisoprolol fumarate

Various proportions of acetonitrile, methanol and phosphate buffer were tested when establishing the optimum mobile phase: 20:30:50, 25:30:45, 30:25:45 and 30:20:50. The most appropriate chromatographic peaks were obtained with a 25:30:45 (v/v/v) mobile phase. Then the influence of the mobile phase flow rate on peak normalization was studied (fig. 3).

The optimum flow rate was 0.3mL/min. The detection was performed at a wavelength of 225nm as it was determined from the absorption spectra of bisoprolol fumarate (fig. 4).

The pH effect on the peak area of a 5µg/mL bisoprolol fumarate sample was examined. Figure 5 shows the pH variation.



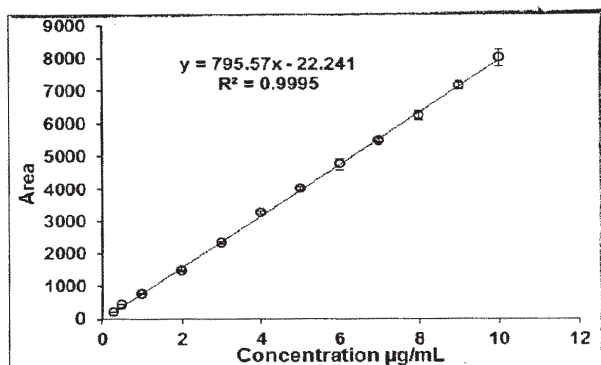


Fig 7. Calibration curve

	$c_{th} = 3\mu\text{g/mL}$		$c_{th} = 5\mu\text{g/mL}$		$c_{th} = 7\mu\text{g/mL}$	
	$C_{exp} (\mu\text{g/mL})$	% nominal	$C_{exp} (\mu\text{g/mL})$	% nominal	$C_{exp} (\mu\text{g/mL})$	% nominal
1	2.96	98.67	5.05	100.98	6.87	98.13
2	3.01	100.33	4.97	99.40	6.93	98.98
3	2.96	98.67	5.07	101.32	6.94	99.12
4	2.99	99.67	5.05	100.99	6.97	99.57
5	3.04	101.33	4.94	98.74	6.99	99.92
6	3.11	103.67	5.10	102.00	7.02	100.29
mean	3.01	100.39	5.03	100.57	6.95	99.34
SD	0.06	1.90	0.06	1.24	0.05	0.77
CV%	1.89	1.89	1.23	1.23	0.75	0.77

$C_{th}$  = theoretical concentration.  $C_{exp}$  = experimental concentration. SD = standard deviation. CV % = coefficient of variation

**Table 2**  
INTRADAY PRECISION AND  
ACCURACY

	$c_{th} = 3\mu\text{g/mL}$		$c_{th} = 5\mu\text{g/mL}$		$c_{th} = 7\mu\text{g/mL}$	
	$C_{exp} (\mu\text{g/mL})$	% nominal	$C_{exp} (\mu\text{g/mL})$	% nominal	$C_{exp} (\mu\text{g/mL})$	% nominal
1	2.89	96.33	4.98	99.60	7.23	103.29
2	2.97	99.00	4.87	97.40	6.97	99.55
3	3.03	101.00	5.03	100.52	6.89	98.48
4	2.93	97.67	5.05	100.99	7.12	101.71
5	3.10	103.33	4.91	98.20	6.99	99.92
6	2.91	97.00	5.11	102.20	7.02	100.29
mean	2.97	99.06	4.99	99.82	7.04	100.54
SD	0.08	2.67	0.09	1.79	0.12	1.71
CV%	2.70	2.69	1.80	1.80	1.71	1.70

$C_{th}$  = theoretical concentration.  $C_{exp}$  = experimental concentration. SD = standard deviation. CV % = coefficient of variation

**Table 3**  
INTERDAY PRECISION AND  
ACCURACY

**Table 4**  
RECOVERY RESULTS OF BISOPROLOL FUMARATE TABLET ASSAY

Concentration added ( $\mu\text{g/mL}$ )	Concentration found mean $\pm$ SD ( $\mu\text{g/mL}$ )	Recovery (%)	RSD (%)
3	2.96 $\pm$ 0.05	98.66	1.68
6	5.98 $\pm$ 0.09	99.79	1.50
9	9.06 $\pm$ 0.13	99.83	1.50

**Table 5**  
ROBUSTNESS STUDY

	Concentration ( $\mu\text{g/mL}$ )	Flow rate ( $\text{mL/min}$ )	Temperature ( $^{\circ}\text{C}$ )	Average area ( $n = 3$ )	Recovery (%)
1	4	0.28	20	3198.87	99.75
2		0.30	25	3201.68	99.85
3		0.32	30	3203.62	100.36
4	5	0.28	20	3913.21	101.08
5		0.30	25	3914.23	99.65
6		0.32	30	3920.02	98.38
7	6	0.28	20	4681.66	100.82
8		0.30	25	4690.28	99.85
9		0.32	30	4687.15	99.69

Recovery and RSD% values (table 4) obtained during bisoprolol fumarate tablets assay showed that the method could be applied with good results in drug control analysis.

To test bisoprolol stability in solutions, a number of 6 replicates with 5 $\mu\text{g/mL}$  concentration of the quality control were used and compared with 6 replicates of stability samples with the same concentration. The solutions were kept at room temperature for 8 h. The obtained results are

summarised in table 6 and showed that bisoprolol is found to be stable in solution for 8 h at room temperature (CV%  $\leq$ 15%).

Analysing the dissolution profiles from figure 8 it was observed that the dissolution percentage of both types of tablets in the specified conditions of USP 29 and at three pH values were higher than 85% (as a percentage of the amount claimed). The average amount of bisoprolol

Analyte		Bisoprolol
Solvent		Mobile phase
Storage conditions		8 hours at room temperature
Comparison Samples		Stability Samples
5µg/mL		5µg/mL
Analyte response		
4021.31		3999.95
3987.45		3978.87
3913.21		3913.21
3967.68		3914.23
3896.41		3920.02
3954.23		3893.41
3977.68		3934.23
4054.52		4013.65
Statistics		
Mean	3971.56	3945.94
SD	52.11	42.17
CV(%)	1.31	1.06

**Table 6**  
ANALYTE STABILITY IN SOLUTION AT ROOM TEMPERATURE

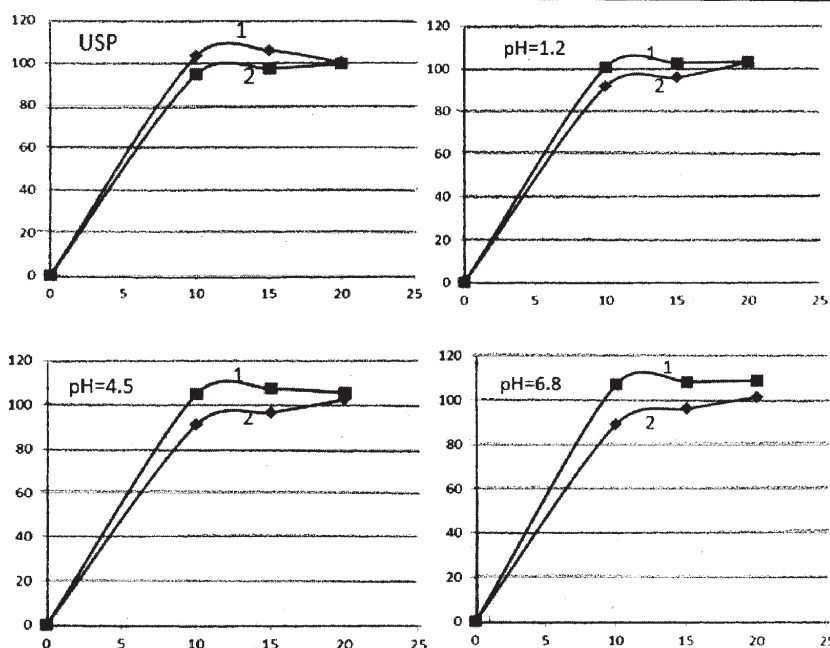


Fig 8. Dissolution profiles for Concor® (1) and Bisotens® (2)

fumarate dissolved within 15 min was higher than 95% for all the tablets (in accordance with USP criteria).

## Conclusions

A sensitive HPLC method with UV detection was developed and validated for bisoprolol fumarate determination from tablets. The purpose of the method was high sensitivity and analysing low concentrations. In the study, the sensibility and recovery percentage of bisoprolol fumarate were higher than those obtained using other methods and the retention time was shorter. The method has demonstrated validity during in vitro bioequivalence study.

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