Quantitative Determination of Bisoprolol Fumarate by HPLC
I. Method validation

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Bisoprolol belongs to the selective beta-blockers class, initially used for the treatment of cardiac arrhythmias and later in patients with arterial hypertension. A simple and accurate method of high performance liquid chromatography (HPLC) was developed and validated for the determination of bisoprolol fumarate in tablets. The chromatographic analysis was performed using an Eclipse XDB C18 type column (150 mm x 4.6 mm, 5 m) with a mobile phase consisting of a mixture of water / methanol / acetonitrile in a ratio of 50:30:20 (v/v/v). The mobile phase flow rate was 1 mL/min, UV detection being carried out at a wavelength of 225 nm. The injected volume was 20 mL. The temperature of the column compartment was kept constant at 25^0 C. The HPLC method for the determination of bisoprolol shows two areas of linearity in the range of 0.8 - 80 g/mL and 80 - 1000 g/mL, respectively. Limit of detection for bisoprolol fumarate was 1.3 g/mL and limit of quantification was 3.98 g/mL.The developed method was appropriately validated for linearity, limit of detection, limit of quantification, precision and accuracy in accordance with the international guidelines. The proposed method proved to be simple, precise, and accurate, and can be successfully applied for the determination of bisoprolol in tablets.

Keywords: bisoprolol fumarate, HPLC, multidiode detector, validation

Hypertension is a complex disease, with numerous cardiovascular complications and beyond, causing a high mortality rate [1]. The prevalence of this disease in Romania ranges from 40% to 44.92% [2]. Bisoprolol, (RS)-1-{4-[(2-isopropoxyethoxy)-methyl]-phenoxy}-3-(isopropylamino)-propan-2-ol (fig. 1) is an antihypertensive drug belonging to the class of selective beta-blockers, initially used for the treatment of cardiac arrhythmias and later in patients with hypertension [3]. Its beneficial effects are due to it action of decreasing the myocardial contractile force and blood pressure. Being less lipophilic, it may cause fewer central nervous system side effects [4].

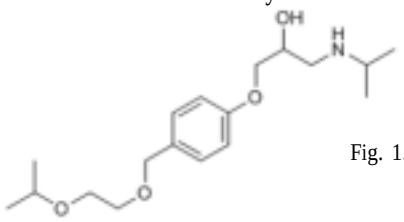

Fig. 1. Structure of bisoprolol

For the determination of bisoprolol in pharmaceutical and biological fluids (plasma, whole blood, urine), alone or in combination with other drugs, numerous analytical methods have been developed. Of these we mention spectrophotometric [5-8] or fluorimetric methods [9, 10], thin-layer chromatographic separation methods [11-14], high performance liquid chromatography with UV detection, fluorescence or mass spectrometry [3, 15-34] and gas chromatography coupled with mass spectrometry [3].

The paper presents the development and validation of a HPLC method for the determination of bisoprolol fumarate in tablets.

Experimental part

Materials and methods

Apparatus and chromatographic conditions

The apparatus used was an Agilent Technologies model 1100 high-performance liquid chromatograph equipped with a multidiode array detector. For sample preparation a Kern 770 analytical balance, an ultrasonic bath and a magnetic stirrer were used. Samples were injected into a chromatographic Eclipse XDB C18 column (150 mm x 4.6 mm, 5 m) at constant temperature of 25^0 C. Separation was performed using a mobile phase consisting of a mixture of water / methanol / acetonitrile in a ratio of 50:30:20 (v/v/v) at a flow rate of 1 mL/min. The injected volume was 20 ml. Detection was performed at 225 nm wavelength.

Reagents

Solvents (acetonitrile, methanol) of chromatographic purity were purchased from Merck's Chemical Co., Darmstadt, Germany. The reference substance, bisoprolol fumarate was obtained from Antibiotice SA Iasi. Tablets containing bisoprolol fumarate were purchased from a local pharmacy.

Standard solution preparation

The standard solution was prepared by diluting 100 mg bisoprolol fumarate (reference substance) in 25 mL

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methanol, and after complete dissolution (about 5 min in an ultrasonic bath) it was diluted with the mobile phase to a volume of 100 mL to obtain a solution with a concentration of 1 mg/mL (1000 g/mL). The thus obtained solution was diluted with the mobile phase to obtain concentrations in the range of 8 to 1000 g/mL. All working solutions were prepared by diluting the stock solution with the mobile phase.

Results and discussion

Method development

The chromatographic column used for the analytical determination was Eclipse XDB C18 (150 mm x 4.6 mm, 5 m). For the analysis of bisoprolol in tablets, during HPLC method development a variety of mobile phases were tested, the most appropriate being the one consisting of water, methanol and acetonitrile in the ratio of 50:30:20 (v/v/v) at a flow rate of 1 mL/min.

The UV spectrum analysis showed a maximum absorption at 225 nm wavelength (fig. 2), value used for all validation tests and practical applications of the method.

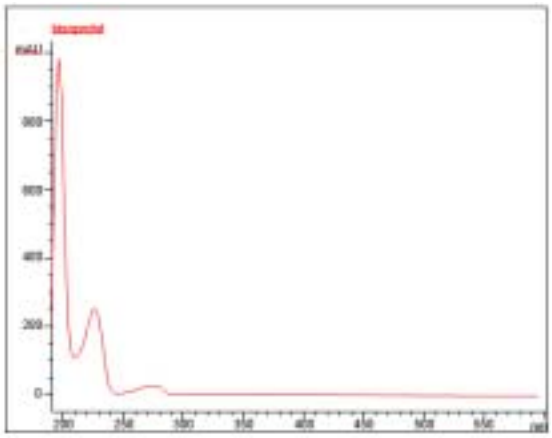

Fig. 2. Absorption spectra of bisoprolol

Method Validation

The developed method was validated in accordance with the requirements of international professional guidelines [35-38]. After establishing the optimal conditions for analysis (stationary phase, mobile phase flow, wavelength detection) the method was validated for the following parameters: bisoprolol identification, linearity, detection limit, quantification limit, precision (system precision, method precision, intermediate precision) and accuracy.

Linearity

For the linearity study three sets of working solutions in a concentration range of 8-1000 g/mL were prepared. Working solutions were obtained by diluting the stock solution with the mobile phase. Each solution was analyzed under the mentioned conditions and from the obtained chromatograms the peak area for bisoprolol was measured. It was found that the HPLC method for the determination of bisoprolol shows two areas of linearity, ranges 0.8 - 80 g/mL and 80 - 1000 g/mL, respectively. Figure 3 shows the right calibration linearity obtained by the method for bisoprolol determination on field work.

In the concentration range 0.8 to 80 g/mL the regression equation was Peak area = 15.745 + 32.184 x concentration (R^2 = 0.9998) and in the concentration range 80-1000 g/mL Peak area = 21.924 x concentration - 605.26 (R^2 = 0.9990).

The linearity of the results was evaluated by graphical representation of the variation in concentration values calculated on the basis of regression line equation

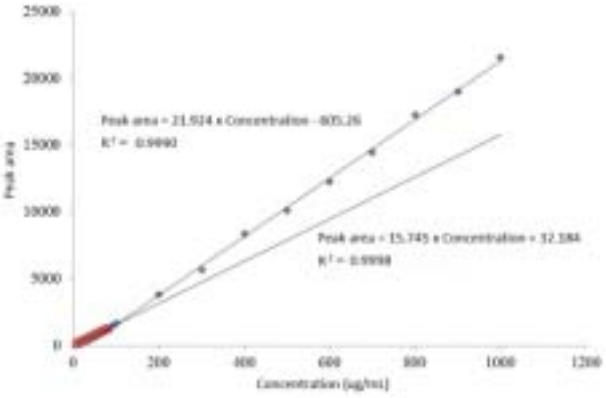

Fig. 3. Calibration curve for the determination of bisoprolol by HPLC

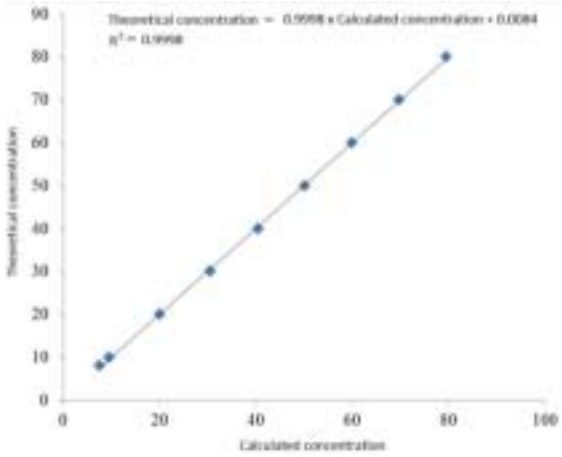

Fig. 4. Linearity results in the determination of bisoprolol by HPLC in the range 8-80 g/mL

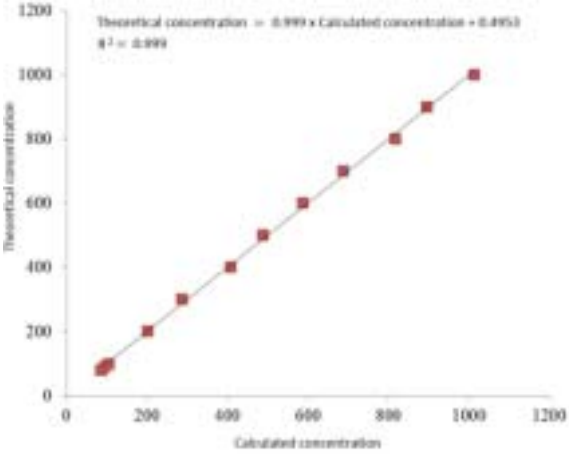

Fig. 5. Linearity results in the determination of bisoprololby HPLC in the range 80 - 1000 g/mL

according to the introduced bisoprolol concentration. The values calculated in the range 8-80 g/mL show a linear slope equal to 0.9998 and intercept 0.0084 with a regression coefficient R^2 = 0.9998. In the range 80-1000 g/mL the slope was 0.999 and intercept 0.4953 with a regression coefficient R^2 = 0.999.

As shown in figures 4 and 5, there was a linear correlation between the theoretical concentration and the concentration calculated using calibration equation there, the slopes of the two straight lines being very close to unity and the intercepts very close to zero.

Limit of detection and the limit of quantification

Limit of detection (LOD = 1.3 g/mL) and limit of quantification (LQ = 3.98 g/mL) were calculated using standard deviation and regression slope.

Precision

To estimate precision, system precision, method precision and intermediate precision were determined. Injection repeatability (system precision) was determined for a number of 10 successive measurements of the same sample, relative standard deviation (RSD) being 0.6602% (maximum limit imposed 2%) (table 1).

Method precision was determined by using three independent solutions in the three different concentration levels for which the relative standard deviation (RSD) was 1.6249% (maximum required 5%) with a confidence interval of the mean value in the range of 98.89% - 101.40% (table 2).

Intermediate precision was determined using three independent solutions at three different concentration levels for which the relative standard deviation (RSD) was 1.6054% (maximum required 5%) with a confidence interval of the mean value in the range 99.29% - 101.78% (table 3).

Accuracy

Accuracy reflects the extent to which a measurement is *close to* the *true value*. The accuracy of the method for determination of bisoprolol was assessed by the addition method. Thus, recovery for three samples at three different concentration levels (in the range of 75 – 125%) was calculated, obtaining a mean recovery of 100.6% in the

Table 1
SYSTEM PRECISION IN THE DETERMINATION OF BISOPROLOL BY HPLC

Determination number	Peak area
1	3786
2	3770
3	3835
4	3830
5	3798
6	3814
7	3812
8	3758
9	3813
10	3787
Average	***3800.3***
SD	***25.0912***
RSD	***0.6602 %***

Table 2
METHOD PRECISION IN THE DETERMINATION OF BISOPROLOL BY HPLC

Det. no.	Theoretical concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	%
1	150	2666	149.21	99.5
2		2615	146.88	97.9
3		2766	153.77	102.5
4	200	3710	196.83	98.4
5		3832	202.39	101.2
6		3879	204.54	102.3
7	250	4821	247.50	99.0
8		4896	250.92	100.4
9		4880	250.19	100.1
Statistical data			**Average**	***100.14 %***
			SD	***1.6273***
			RSD	***1.6249 %***

Table 3
INTERMEDIATE PRECISION IN THE DETERMINATION OF BISOPROLOL BY HPLC

Det. no.	Theoretical concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	%
1	15	2740	152.58	101.7
2		2616	146.93	98.0
3		2742	152.68	101.8
4	20	3698	196.28	98.1
5		3877	204.45	102.2
6		3844	202.94	101.5
7	25	4930	252.47	101.0
8		4842	248.46	99.4
9		4937	252.79	101.1
Statistical data			**Average**	***100.53 %***
			SD	***1.6140***
			RSD	***1.6054 %***

Det. no.	Theoretical concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	Recovery %
1	15	2758	153.41	102.3
2		2770	153.95	102.6
3		2773	154.09	102.7
4	20	3762	199.20	99.6
5		3787	200.34	100.2
6		3714	197.01	98.5
7	25	4834	248.10	99.2
8		4812	247.09	98.8
9		4934	252.66	101.1
Statistical data			**Mean recovery**	***100.6 %***
			Minimum	***98.5 %***
			Maximum	***102.7 %***

Table 4
ACCURACY IN THE DETERMINATION OF BISOPROLOL BY HPLC

range 98.5 - 102.7%. Since the recovery method has a value within the specified range (± 5%) it follows that the method is accurate (table 4).

Conclusions

The developed and validated HPLC method is simple, precise, and accurate, and was successfully applied to determine bisoprolol in tablets, being sufficiently sensitive. The proposed method can be used in quality control, routine analysis of the tablets or in the study of stability of bisoprolol-containing tablets.

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