SPECTROPHOTOMETRIC DETERMINATION OF BISOPROLOL USING METHYL ORANGE AS REAGENT

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Abstract

A spectrophotometric method for the quantitative determination of bisoprolol was established based on the formation of an ion pair complex between bisoprolol and methyl orange, in acid medium, extractable in dichloroethane, with a maximum absorbance at 427 nm. The limit of detection (LoD) was 0.20 µg/mL and the limit of quantification (LoQ) was 0.66 µg/mL. The elaborated method was validated.

Rezumat

Pentru determinarea bisoprololului fumarat s-a dezvoltat o metodă spectrofotometrică, bazată pe formarea unui complex de tip perechi de ioni între bisoprolol şi metil orange în mediu acid, extractibil în dicloretan, cu un maxim de absorbţie la 427 nm. Limita de detecţie (LD) a fost de 0,20 µg/mL, iar limita de cuantificare (LC) a fost 0,66 µg/mL. Metoda elaborată a fost validată.

Keywords: Bisoprolol, VIS spectral method, assay, methyl orange.

Introduction

Bisoprolol is a valuable drug used in the treatment of cardiovascular diseases. It has special pharmacokinetic properties emphasized by clinical studies.

There are few spectrophotometric methods for the assay of beta blockers and fewer for bisoprolol [1,2,3,5,7]. It should be noted that the actual spectrophotometric methods used for bisoprolol determination are UV-based.

This paper presents a new VIS spectrophotometric method for the assay of bisoprolol using methyl orange as reagent. The developed method was validated [4,6,8,9] using pure substance and pharmaceutical tablets.

Materials and Methods

Reagents: Bisoprolol fumarate –100.07% pure reference substance, Unichem Laboratories LTD, India; Glacial acetic acid p.a. – Tunic Prod, Romania; Methyl orange – *Reactivul Bucureşti*, Romania; Dichloroethane

p.a. – Fluka, Germany; Monosodium phosphate (NaH_2PO_4) p.a, disodium phosphate dihydrate ($Na_2HPO_4 \times 2H_2O$) p.a - *Reactivul Bucureşti*, Romania.

Equipment: Analytical balance (Kern 770); UV-VIS spectrophotometer (Hewlett Packard 8453); Ultrasonic bath; Ika Vibrax VXR Basic.

Solutions:

1. Stock solution (100 μg/mL): 10 mg bisoprolol (100.07 % pure reference substance) was dissolved in 100 mL of 2M acetic acid in a volumetric flask;
2. Working solutions containing from 0.8 to 9 μg/mL bisoprolol were obtained by diluting the stock solution with 2M aqueous solution of acetic acid;
3. Aqueous solution of methyl orange, 0.1% (w/v);
4. Aqueous solution of acetic acid, 2M;
5. Phosphate buffer, pH = 7.4, prepared according to Romanian Pharmacopoeia – 10^{th} edition [4].

In order to establish the optimum wavelength for the detection, 1 mL of 6 μg/mL working solution was mixed with 0.5 mL of 0.1% methyl orange solution and 1 mL phosphate buffer of pH = 7.4. After mixing, the obtained complex was three times extracted using 2.5 mL dichloroethane. The UV-VIS absorption spectra using 1 cm cell were recorded for reaction product obtained after 15 minutes.

In order to establish the optimum working conditions, two solutions of 0.8 μg/mL and 9 μg/mL were used (the minimum and maximum concentration of the linear range), while the parameters of the method were changed. The optimum concentration of the methyl orange solution, the pH value and the stability of the reaction product were established.

Procedure: 0.5 mL of 0.1 % (w/v) methyl orange solution was added to 1 mL acetic solution containing bisoprolol in a concentration range of 0.8 and 9 μg/mL, followed by the addition of 1 mL phosphate buffer of pH = 7.4. This mixture was shaken for five minutes and than the obtained complex was three times extracted using 2.5 mL dichloroethane. The absorbance was measured at 427 nm (1cm cell) *versus* a blank solution prepared in similar conditions.

Validation of the method

Linearity: solutions containing bisoprolol in a concentration range of 0.5 – 16 μg/mL were used. Detection limits (LoD) and quantification limits (LoQ) were calculated using the following formulas [7]:

$$LOD = \frac{3 \times SE}{Slope}, \quad LOQ = \frac{10 \times SE}{Slope},$$

where SE is the standard error of the linear regression.

Precision: the method precision was investigated through its repeatability and reproducibility. Three solutions of 4 µg/mL, 5 µg/mL and 6 µg/mL bisoprolol were used. Three assays were performed for each concentration in the same day in order to evaluate the repeatability. Also, three sets of assays were performed in different days in order to evaluate the reproducibility.

Accuracy: in order to establish the accuracy of the method, bisoprolol solutions of 4 µg/mL, 5 µg/mL and 6 µg/mL were analyzed. For each concentration, three determinations were performed.

Results and Discussion

From the analysis of the absorption spectra (figures 1, 2, 3), a maximum absorbance was observed for the reaction product at 427 nm (figure 3). This value was used for all the determinations. As can be seen in figure 2 that bisoprolol in 2M acetic acid solution presented a maximum absorbance at 438 nm. The specific absorption coefficient of bisoprolol was $A^{1\%}_{1cm,438nm} = 2.41$ and the specific absorption coefficient of the reaction product was $A^{1\%}_{1cm,427nm} = 802$.

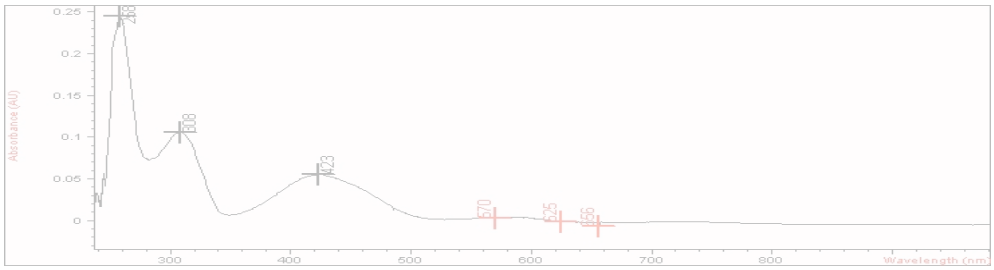

Figure 1
Absorption spectra of methyl orange (solution 0.1%)

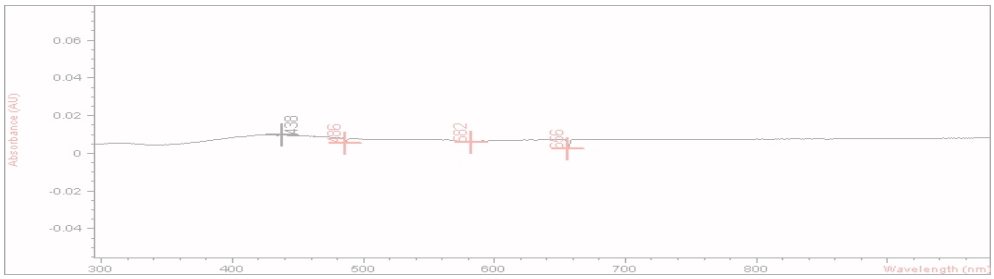

Figure 2
Absorption spectra of bisoprolol fumarate (6µg/mL)

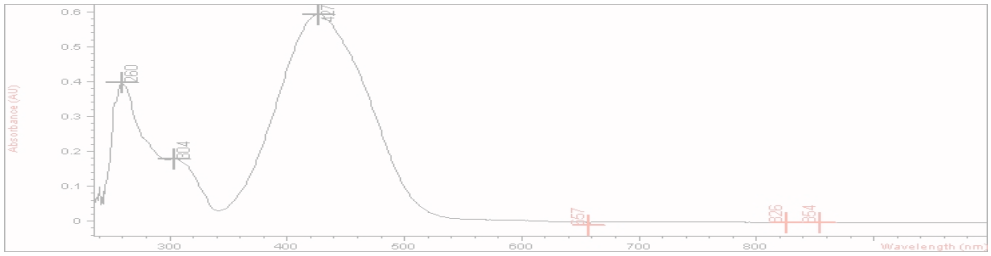

Figure 3
Absorption spectra of the complex methyl orange – bisoprolol (6µg/mL)

The influence of the concentration of the reagents used for the preparation of the working solutions on the complex stability was also investigated. According to table I, the optimal reagents concentrations were 2M for the acetic acid solution and 0.1% (w/v) for the methyl orange solution, respectively.

Bisoprolol reaction with methyl orange was also investigated at different pH values. As it can be seen in table I, an optimal stability of the formed complex was obtained at pH = 7.4.

Table I
Reagents concentration

Acetic acid (M)	Concentration of bisoprolol		Methyl orange (%)	Concentration of bisoprolol		Phosphate buffer pH	Concentration of bisoprolol	
	0.8 µg/mL	9 µg/mL		0.8 µg/mL	9µg/mL		0.8 µg/mL	9µg/mL
0.5	0.0439	0.8476	0.025	0.0387	0.8449	5.29	0.0433	0.8486
1.0	0.0440	0.8495	0.05	0.0398	0.8502	6.26	0.0437	0.8497
1.5	0.0445	0.8498	**0.1**	**0.0454**	**0.8522**	6.64	0.0449	0.8534
2.0	**0.0455**	**0.8504**	0.5	0.0452	0.8521	**7.40**	**0.0453**	**0.8543**
2.5	0.0452	0.8501	1	0.0451	0.8504	7.79	0.0451	0.8533
3.0	0.0450	0.8494	1.25	0.0451	0.8502	8.32	0.0447	0.8524

The absorbance was measured 15 minutes after the last extraction with dichloroethane (table II).

Table II
Complex stability in time

Concentration of bisoprolol (µg/mL)		Time (minutes)						
		10	15	20	25	30	40	60
0.8	Absorbance	0.0451	**0.0456**	0.0454	0.0452	0.0453	0.0449	0.0448
9		0.8543	**0.8549**	0.8526	0.8523	0.8521	0.8503	0.8495

Linearity: the obtained data (table III) were analyzed by linear regression and the calibration curve was obtained (figure 4). According to

the experimental data, the developed method for bisoprolol determination was linear in the concentration range of 0.8 - 9 µg/mL. The corresponding linearity parameters are presented in table III.

The following calibration curve equation was established:

Absorbance = 0.0991 × concentration – 0.033

Table III
Method linearity

Concentration of bisoprolol (µg/mL)	Absorbance				Mean
	I	II	III	IV	
0.5	0.0271	0.0399	0.0404	0.0406	0.0370
0.8	0.0453	0.0457	0.0462	0.0455	0.0457
1.0	0.0735	0.0691	0.0634	0.0645	0.0657
2.0	0.1637	0.1626	0.1617	0.1627	0.1624
3.0	0.2644	0.2650	0.2643	0.2644	0.2646
4.0	0.3587	0.3579	0.3519	0.3588	0.3562
5.0	0.4644	0.4639	0.4643	0.4644	0.4642
6.0	0.5721	0.5698	0.5698	0.5711	0.5702
7.0	0.6709	0.6709	0.6698	0.6718	0.6708
8.0	0.7587	0.7578	0.7579	0.7609	0.7589
9.0	0.8498	0.8488	0.8487	0.8477	0.8484
10	0.8469	0.8465	0.8865	0.8458	0.8596
11	0.8468	0.8451	0.8648	0.8434	0.8511
12	0.8473	0.8468	0.8538	0.8467	0.8491
13	0.8428	0.8432	0.8458	0.8442	0.8444
14	0.8387	0.8375	0.8365	0.8368	0.8369
15	0.8356	0.8348	0.8354	0.8346	0.8349
16	0.8357	0.8344	0.8357	0.8351	0.8350
Linearity parameters	Range 0.8 - 9 µg/mL, Intercept = -0.0333, Slope = 0.0991, Correlation coefficient r = 0.9997, detection limit (LOD) = 0.20 µg/mL, quantification limit (LOQ) = 0.66 µg/mL				

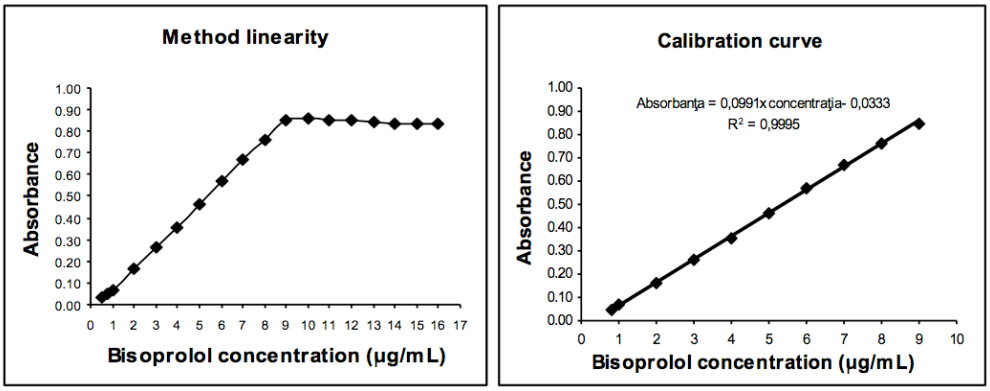

Figure 4
Method linearity and calibration curve

Method precision: the sample concentration was calculated using the calibration curve equation, which was obtained on the basis of experimental data as above indicated (table IV). According to table IV, the relative standard deviation for all sets of data was lower than 2% (RSD = 1.07), which proved that the proposed method was precise.

Table IV

Method precision and accuracy

Concentration of bisoprolol (μg/mL)	Repeatability		Reproducibility		Accuracy	
	Absorbance	Recovery %	Absorbance	Recovery%	Absorbance	Recovery%
4	0.3585	98.82	0.3612	99.50	0.3584	98.80
4	0.3599	99.18	0.3597	99.13	0.3589	98.93
4	0.3597	99.13	0.3698	101.67	0.3598	99.15
5	0.4691	101.37	0.4701	101.57	0.4701	101.57
5	0.4698	101.51	0.4687	101.29	0.4696	101.47
5	0.4675	101.05	0.4660	100.75	0.4648	100.50
6	0.5634	100.33	0.5721	101.79	0.5688	101.24
6	0.5675	101.02	0.5597	99.71	0.5621	100.11
6	0.5668	100.90	0.5738	102.08	0.5678	101.07
Statistical data	Mean = 100.36 RSD = 1.11		Mean = 100.83 RSD = 1.04		Mean = 100.31 RSD = 1.07	

Accuracy: the concentration of the sample was calculated from the experimental values of the absorbance, using the regression curve equation (table IV). In all cases, a recovery degree ranging between 98.80 and 101.57 % and a relative standard deviation under 2% (RSD = 1.07) were obtained for the studied concentration range, which in turn prove that the proposed method is accurate.

Determination of bisoprolol fumarate in pharmaceutical tablets

The method developed for the spectrophotometric determination of bisoprolol in VIS was applied for the investigation of some pharmaceutical tablets from 3 different manufacturers. A number of 20 tablets containing bisoprolol were crushed in a mortar, than an equivalent quantity of about 5 mg bisoprolol was taken from the obtained powder and dissolved in 100 mL of distilled water under agitation on an ultrasonic bath. The obtained solution was filtered in order to separate the excipients and totally evaporated, than the dry matter was dissolved into a volumetric flask of 100 mL using acetic acid. Samples of 0.3 mL, 0.5 mL and 0.8 mL were taken from the obtained solution and adjusted with 2M acetic acid up to a standard volume of 1 mL. Then, 0.5 mL methyl orange solution of 0.1% and 1 mL phosphate buffer of pH=7.5 were added. The obtained mixture was

extracted with 7.5 mL dichloroethane. Three successive weightings were performed.

The following experimental results were obtained:

- Concor® (Merck) – 5 mg/tablet – recovery of 99.1-101%;
- Bisotens® (*Antibiotice Iasi*) – 5 mg/tablet – recovery of 99.8-100.9%;
- Bisoblock® (Keri Pharma Generics Ltd) - 5 mg/tablet – recovery of 98.9-101.70 %

These recovery tests are in accordance with those obtained using the reference substance. According to the average experimental values, the bisoprolol content (mg/tablet) is ranging within the limits imposed by the Romanian Pharmacopoeia – 10th edition (for the active substance content, a percent experimental error of ±10 % is accepted). Overall, the above experimental results confirm the repeatability and reproducibility of the proposed analysis method.

Conclusions

In this paper, the elaboration and the testing of a VIS spectrophotometric method is described. The spectrophotometric method was developed for the assay of bisoprolol using methyl orange as reagent in acid medium. The proposed method is based on the formation of a specific colored product and, at our knowledge, is not available in the literature. The color reaction is induced in the presence of an amino group from the bisoprolol molecule; consequently, the ingredients interference is practically eliminated. The reaction product, stable at pH = 7.4 and extracted with dichloroethane, shows a maximum absorbance at 427 nm. The specific absorption coefficient of bisoprolol in acetic acid and of the corresponding reaction product were: $A^{1\%}_{1cm,438nm} = 2.41$ and $A^{1\%}_{1cm,427nm} = 802$. The measurement detection sensitivity significantly increased (330 fold). The analytical method was optimized and validated by establishing the linearity (in the range 0.8 - 9 μg/mL), the correlation coefficient (r = 0.9997), the detection limit (0.20 μg/mL), precision (RSD = 1.07), and the accuracy (mean recovery = 100.31).

Thus, the particularity of the developed method consists in its enhanced sensibility, in comparison with other available UV spectrophotometric methods. The developed method is simple and easy to be performed, which are common advantages of the spectrophotometric methods (1,5,7).

The experimental results concerning the recovery of bisoprolol in tablets were in accordance with those obtained using the reference substance. According to the average experimental values, the bisoprolol content (mg/tablet) is ranging within the limits imposed by Romanian Pharmacopoeia – 10th edition.

The method has a high potential to be applied in control quality laboratories, for routine analysis of the pharmaceutical forms, where cheap and fast measurements are essential.

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