A New Method for the Assay of Bisoprolol Using Bromocresol Green

ALINA DIANA PANAINTE, NELA BIBIRE*, GLADIOLA TANTARU, MIHAI APOSTU, MADALINA VIERIU, VASILE DORNEANU

“Grigore T. Popa” University of Medicine and Pharmacy, Faculty of Pharmacy, Analytical Chemistry Department, 16 University Str., 700115, Iasi, Romania

A new Vis spectrophotometric method was developed for the assay of bisoprolol in pharmaceutical preparations using bromocresol green in hydrochloric acidic medium. The reaction product showed a maximum absorbance at 402 nm proportional with the concentration of bisoprolol. The optimum conditions for the reaction were established. The developed method was validated. The method showed a good linearity in the concentration range of 7 – 80 μg/mL (correlation coefficient r = 0.9998). The detection limit (LOD) was 1.78 μg/mL and the quantification limit (LOQ) was 5.41 μg/mL. The precision and the accuracy were determined; mean recovery was 100.11% in the 98.35-101.57% concentration range.

Keywords: bisoprolol, bromocresol green, spectrometric method, validation.

Bisoprolol fumarate (±)-1-{p-[2-(isopropoxyethoxy) methyl] phenoxy}-3-isopropyl-amino-2-propanol hemifumarate is a beta1-selective (cardioselective) adrenoceptor blocking agent without significant membrane stabilizing or intrinsic sympathomimetic activities in its therapeutic dose range. It is prescribed for the treatment of hypertension and angina pectoris [1, 2]. The S(-) enantiomer is responsible for most of the beta-blocking activity. The structural formula is presented in figure 1.

![Chemical structure of bisoprolol fumarate (2:1)](image)

In comparison with other beta-selective blockers (atenolol, metoprolol, betaxolol) bisoprolol proved to be the compound with the highest beta-selectivity in all in vitro and in vivo experiments and in all animal species investigated [3-8].

Several methods have been reported for the determination of bisoprolol in plasma and urine samples: high-performance liquid chromatography (HPLC) [9-14], liquid chromatography–tandem mass spectrometry (LC–MS-MS) [15-18], liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) [19-20] and electrophoresis [21]. Spectrophotometric methods UV-Vis absorption are fast, robust, precise and universally accepted in pharmaceutical analysis [22]. Most spectrophotometric methods for bisoprolol determination are in UV range. In literature there are few methods for the analysis of bisoprolol in visible domain that include reactions with either 7,7,8,8-tetracyano quinodimethane [23], tropaeolin 000 [24] or chromazurol S [24] with maximum absorption at 587.5 nm, 485 nm and 505 nm respectively.

The aim of the present investigation was to develop a new rapid and reproducible method for the quantitative determination of bisoprolol fumarate in pharmaceutical products. The method was based on the formation of blue coloured ion pair complex between bisoprolol and bromocresol green in acidic medium, which was extracted in chloroform and then spectrophotometrically analyzed at 402 nm (λmax).

**Experimental part**

**Materials and method**

Absorbance was measured in quartz cuvettes using a Hewlett Packard 8453 UV–Vis spectrophotometer at the room temperature. An ultrasonic bath and a vibration shaker IKA-Werke type VX2 were also used.

The reagent used were: bisoprolol fumarate – 100.07% pure reference substance, Unichem Laboratories LTD, India; chloroform - Fluka, Germany; hydrochloric acid - Tunic Prod, Romania; bromocresol green - Tunic Prod, Romania.

The pharmaceutical products Concor® (Merck), Bisotens® (Antibiotice iasi) and Bisoblock® (Keri Pharma Generics Ltd) have been purchased from the local pharmacies.

A 100 μg/mL stock bisoprolol solution was prepared and diluted to obtain standard solutions of various concentrations.

In order to establish the optimum working conditions, two solutions of 7 μg/mL and 80 μg/mL were used (the minimum and maximum concentration of the linear range), while the parameters of the method were modified. The optimum concentration of bromocresol green solution and hydrochloric acid solution were established. The most suitable solvent has been established for the extraction. The stability of the reaction product was studied. The validation of the proposed method was done according to ICH guidelines [25]. The following parameters have been studied: linearity, detection and quantification limit, system method precision, the accuracy and robustness of the method [26-29].

**Procedure for pharmaceutical formulation**

The developed method was applied for the assay of bisoprolol in three pharmaceutical products. Twenty bisoprolol tablets for each product were crushed into a mortar, then a quantity of powder equivalent to 5 mg bisoprolol was stirred in 50 mL methanol on an ultrasonic bath. Afterwards the solution was filtered and then it was evaporated to dryness. The residue was dissolved into a

*email: nelabibire@yahoo.com*
100 mL volumetric flask using distilled water. A 20 μg/mL solution was obtained through dilutions. Various concentrations were obtained through dilution with distilled water. Then 1 mL sample was processed according to the working procedure.

**Assay procedure**

1 mL 0.1 M hydrochloric acid and 1 mL 0.01% (w/v) bromocresol green aqueous solution were added to 1.0 mL of bisoprolol fumarate solution with a concentration between 7-80 μg/mL. The complex was then extracted using chloroform. Ten minutes later the absorbance was measured at 402 nm, using as reference a blank sample prepared in the same conditions.

**Results and discussions**

From the analysis of the absorption spectra (fig. 2), a maximum absorbance was observed for the reaction product at 402 nm. This value was used for all the determinations.

As can be seen in figure 3, bisoprolol and bromocresol green presented maximum absorbance peaks at 275 nm and 615 nm, respectively. The specific absorption coefficient of bisoprolol was $A_{1\% 1cm,402nm} = 250$ and the specific absorption coefficient of the reaction product was $A_{1\% 1cm,402nm} = 960$.

Comparing the spectra from figure 4, it was observed that the proposed method was selective because it had the ability to separate the corresponding signal of bisoprolol.

The excipients used in the pharmaceutical formulation did not interfere with the maximum absorption of bisoprolol.

The stoichiometric ratio of the reaction product was investigated by Job’s continuous variation method. For that study, $1 \times 10^{-2}$ M bromocresol green and bisoprolol solutions were used.
were mixed in varying volume ratio while the total volume of the mixtures was maintained constant at 10 mL.

From the analysis of figure 5, the optimal combination ratio of the bisoprolol and bromcresol was established to be 1:1.

According to table 1, the optimal concentrations of reagent were 0.1 M for the hydrochloric acid solution and 0.01% (w/v) for the bromocresol green solution.

The stability of the reaction product was evaluated at room temperature without protection from light. The absorbance was measured after 10 min extraction in chloroform according to the experimental data. From figure 6, the absorbance remained almost the same for at least 15 min, time sufficient enough for the analysis to be performed.

While choosing the best solvent of the extraction, experiments were done with ethanol, methanol, chloroform, dicloromethan, acetonitrile (fig. 7). It has been observed that the best results were obtained when the extraction was performed in chloroform.

Linearity was assessed by analyzing the obtained data shown in table 2 by linear regression and the calibration curve from figure 8 was obtained.

The following calibration curve equation was established:

\[
\text{Absorbance} = 0.012 \times \text{Concentration} + 0.0034
\]

The parameters obtained for the validation of the method are summarized in table 3.

Detection (LOD) and quantification limits (LOQ) were calculated using the following equations [28]:

Table 1

<table>
<thead>
<tr>
<th>INFLUENCE OF REAGENT CONCENTRATION</th>
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<tr>
<td>Hydrochloric acid</td>
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<td>0.0808</td>
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Table 2

<table>
<thead>
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<th>LINEARITY STUDY</th>
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<tr>
<td>Concentration (µg/mL)</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>3</td>
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Fig. 6. Stability of the reaction product

Fig. 7. Choice of solvent for the extraction
Table 6
COMPARISON WITH OTHER SPECTROPHOTOMETRIC METHODS

| LOD = 3.3 x Standard error / Slope = 1.78 μg/mL |
| LOQ = 10 x Standard error / Slope = 5.41 μg/mL |

For a 40 μg/mL sample ten determinations were done under the same experimental conditions for the study of the suitability of the system. The relative standard deviation (RSD = 0.67%) showed that the system was precise.

Three different concentration solutions of bisoprolol were used thrice for the determination of the precision and accuracy of the method. The method precision was investigated through its repeatability and intermediate precision. Standard addition method was used to evaluate the accuracy of the method. The concentrations of the samples were calculated using the equation of the calibration curve (Table 4). It was observed that the relative standard deviation was lower than 2% and thus the method was precise. Because the recovery was 100.11% it was concluded that the method was also accurate.

The evaluation of robustness was done by changing the instrument, and varying the volume of bromocresol green and the concentration of hydrochloric acid. Reproducibility of the results confirmed the robustness of the method.

Fig. 8. Calibration curve
The proposed method has been applied for the analysis of bisoprolol in tablets. For each sample three determinations were done. The results listed in table 5 showed that the bisoprolol content (mg/tablet) was ranging within the limits imposed by 10th Edition Romanian Pharmacopoeia – [29]. Excipients were found not to interfere in the analysis.

The new method was compared with other spectrophotometric methods. It was observed that the results were similar to those already published (table 6).

Conclusions

A new spectrophotometric method was developed for the assay of bisoprolol using bromocresol green as reagent in acidic medium. The proposed method was based on the formation of a specific colored product. The reaction product extracted in chloroform showed a maximum absorbance peak at 402 nm. The specific absorption coefficient of bisoprolol and that of the corresponding reaction product were: $A_{402 nm}^{1%\text{mg/mL}} = 250$ and $A_{402 nm}^{1%\text{ug/mL}} = 960$.

The analytical method was optimized and validated by establishing the linearity domain (in the concentration range 7 - 80 μg/mL), the correlation coefficient ($r = 0.9998$), the detection limit (1.78 μg/mL), precision (RSD = 1.06) and the accuracy (mean recovery = 100.11). The experimental results concerning the recovery of bisoprolol in tablets were in accordance with those obtained using the reference substance.

The main advantage of the new method consists in its enhanced sensibility, in comparison with other UV spectrophotometric methods. The new method was simple and easy to perform and it may be used for quality control or for routine analysis of pharmaceutical products when cheap and fast measurements are essential.

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References


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