SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BISOPROLOL FUMARATE IN TABLETS

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SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF BISOPROLOL FUMARATE IN TABLETS (Abstract): Bisoprolol fumarate is prescribed for the treatment of hypertension and angina pectoris. **Aim:** The purpose of this study was to develop a simple, sensitive, accurate, and reproducible method for estimation of bisoprolol fumarate in tablets. **Material and methods:** The proposed method was based on a yellow colored complex formed with tropaeolin 00, extractable in dichloromethane with maximum absorbance at 412 nm. The method was validated statistically. **Results:** The linearity domain was observed in the concentration of 5-30 µg/ml. The recovery studies confirmed the accuracy of the proposed method. **Conclusions:** The proposed method can be applied for the routine analysis of bisoprolol from formulations. **Keywords:** BISOPROLOL FUMARATE, SPECTROPHOTOMETRIC METHOD, TROPAEOLIN 00.

Bisoprolol is a β blocker cardiovascular drug. It is structurally similar to metoprolol, acebutolol and atenolol in that it has two substituents in the para position of the benzene ring (fig.1) which might be the reason for its β1-selectivity.

![Fig. 1. The structural formula of bisoprolol fumarate](image)

Bisoprolol is used for the treatment of hypertension, cardiac ischemia and congestive heart failure. This is also indicated for preventative treatment before and primary treatment after heart attacks thus reducing the chances of recurrence (1). Bisoprolol reduces the high blood pressure in hypertension, while in cardiac ischemia this drug is used to reduce the activity of the heart muscle and therefore reduce oxygen and nutrient demand, so reduced blood supply can still transport sufficient amounts of oxygen and nutrients (2-6).

Various methods such as UV-Vis spectrophotometry (7-10), high-performance liquid chromatography (11-13), and thin layer chromatography (14-16) are available for determination of bisoprolol in biological fluids and in pharmaceutical formulations.

The aim of the present work was to de-
Develop a new rapid and reproducible spectrophotometric method for the quantitative determination of bisoprolol fumarate in pharmaceutical products.

**MATERIAL AND METHODS**

A Hewlett Packard 8453 double beam UV–Vis spectrophotometer equipped with 10 mm matched quartz cells was used in the present investigation. A Kern 770 analytical balance, 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), hydrochloric acid (Tunic Prod, Romania), dichloromethane (Fluka, Germany); tropaeolin 00 (Tunic Prod, Romania).

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

Preparation of the solutions:

- Stock solutions of bisoprolol fumarate was prepared by dissolving accurately weighed 10 mg of standard substance in a 100 mL volumetric flask with distilled water;
- Standard solutions of various concentrations of bisoprolol were prepared by suitable dilution from stock solutions;
- 0.01% (m/v) aqueous solution of tropaeolin 00: 0.01 g of tropaeolin 00 was dissolve on ultrasonic bath in 100 mL volumetric flask with distilled water;
- 2M aqueous solution of hydrochloric acid was prepared by transferring 3.8 mL hydrochloric acid (96%) in 50 mL volumetric flasks and bringing up to the mark with distilled water;
- Sample solution: twenty tablets of each formulation were accurately weighed and the average weight was calculated for each. These tablets were grounded to a fine powder. An accurately weighed tablet powder equivalent to 5 mg of bisoprolol fumarate was mixed with 30 mL of methanol and sonicated for 30 minutes. Insoluble excipients were separated through filtration. Then the solution was evaporated to dryness. The residue was dissolved into a 100 mL volumetric flask using distilled water. The obtained solution was diluted with distilled water to obtain working standard solution corresponding to 20 µg/ml.

Assay procedure: 1 mL 0.05 M hydrochloric acid and 1 mL 0.01% (w/v) tropaeolin 00 aqueous solution were added to 1 mL of bisoprolol fumarate solution with a concentration in between 5-30 µg/mL. The complex was then extracted using dichloromethane. Fifteen minutes later the absorbance was measured at 412 nm, using as reference a blank sample prepared in the same conditions.

Validation: The method was validated with respect to linearity, accuracy, precision and LOD and LOQ (17).

**RESULTS AND DISCUSSION**

The analysis of the absorption spectra (fig. 2) revealed a maximum absorbance of the reaction product at 412 nm. This value was used for all determinations.

As can be notice in fig. 3, bisoprolol in dichloromethane solution presented a maximum absorbance peak at 385 nm and tropaeolin 00 had no absorption peaks in the interest range.

Comparing the spectra from fig. 3 and 4, it was observed that the proposed method was selective because it had the ability to separate the signal of bisoprolol from the one corresponding to the reaction product.
The excipients used in the pharmaceutical formulation did not interfere the maximum absorption of bisoprolol complex.

While choosing the best solvent of the extraction, experiments were done using ethanol, methanol, chloroform, dichloromethane, acetonitrile (fig. 5). It has been observed that the best results were obtained when the extraction was performed using dichloromethane.
Spectrophotometric method for estimation of bisoprolol fumarate in tablets

The calibration curves for bisoprolol fumarate was founded to be linear in the concentration range of 5-30 μg/mL. The optical and regression characteristics of the drugs are show below (tab. I).

The absorbance of each tablet formulations was measured and the results of analysis were shown in tab. II.

Accuracy: To study the accuracy of the proposed method, recovery studies were carried out by adding a known amount of drug to the pre analyzed tablet powder and percentage recoveries were calculated. The result of recovery studies are presented in tab. III.

Precision: The reproducibility of the proposed method was determined by performing the analysis at different time moments in the same day (intra-day assay precision) and three different days (inter-day assay precision). The results of intra-day and inter-day precisions expressed in relative standard deviation percentages were found to be 0.8 and 0.9, respectively.

Limit of detection and limit of quantification: The LOD and LOQ were determined based on the standard deviation of the intercept and the slope of the calibration curves. LOD and LOQ for bisoprolol were found to be 0.67 μg/mL and 2.23 μg/mL respectively.

**TABLE I**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>412</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range, μg/mL (μg/ml)</td>
<td>5-30</td>
</tr>
<tr>
<td>Molar Absorptivity (1/mol·cm)</td>
<td>$1.03 \cdot 10^4$</td>
</tr>
<tr>
<td>Sandell’s Sensitivity (mg/cm²/0.001 absorbance unit)</td>
<td>0.0134</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.0029</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Label Claim (mg)</th>
<th>Amount of Estimated Drug (mg)</th>
<th>Label Claim Percentage (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concor®</td>
<td>2.5</td>
<td>2.49</td>
<td>99.33</td>
<td>0.59</td>
</tr>
<tr>
<td>Bisotens®</td>
<td>5</td>
<td>4.96</td>
<td>99.86</td>
<td>0.67</td>
</tr>
<tr>
<td>Bisoblock®</td>
<td>10</td>
<td>10.03</td>
<td>100.31</td>
<td>0.13</td>
</tr>
</tbody>
</table>
### TABLE III

**Result of recovery studies**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Bisoprolol in tablet (µg/mL)</th>
<th>Pure Drug Added (µg/mL)</th>
<th>Recovery* Percentage (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concor®</td>
<td>12</td>
<td>8</td>
<td>99.15</td>
<td>0.42</td>
</tr>
<tr>
<td>Bisotens®</td>
<td>15</td>
<td>5</td>
<td>98.87</td>
<td>0.96</td>
</tr>
<tr>
<td>Bisoblock®</td>
<td>17</td>
<td>3</td>
<td>99.23</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Mean for 3 determinations

### CONCLUSIONS

The presented assay provides a simple, accurate, economical and convenient method for the analysis of bisoprolol using VIS spectrophotometry. Results of percentage recovery showed that the method was not influenced by excipients.

The method was validated and its sensitivity and precision proved the suitability of the proposed method for the routine estimation of bisoprolol in pharmaceutical formulations.

### ACKNOWLEDGEMENTS

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### REFERENCES


NEW PROPERTIES OF SOME SUGARCANE CULTIVARS

The phenolic composition, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*) were investigated by Abbas et al. Sugarcane is a dietary source of flavonoids and it is used as functional food in many countries. In addition, it is an important cash crop of Pakistan. The sugarcane leaves are the main raw material for the sugar industry. The authors studied the juices and the water extracts obtained from the leaves of thirteen varieties of sugarcane. The antioxidant activity of sugarcane extracts and juices was evaluated using DPPH radical scavenging assay. In this assay, the extracts showed better antioxidant activity than the juices. The IC$_{50}$ values ranged from 20.82 to 27.47 μg/mL for the leaves extracts and from 63.95 to values higher than 200 μg/mL for the juices. In the phosphomolybdenum assay, the juices showed a better electron-donating activity than the extracts. In this assay, the IC$_{50}$ values ranged between 62.3 and 80.26 μg/mL (extracts) and 70.43 and 99.9 μg/mL (juices). The leaves extracts and juices showed strong ability to protect against DNA damage induced by hydroxyl radical generated in Fenton reaction. The high performance liquid chromatography (HPLC) analysis of extracts was also performed. Ferulic acid (14.63 ± 0.03 mg/g), eumaric acid (11.65 ± 0.03 mg/g), quercitrin (10.96 ± 0.02 mg/g), caffeic acid (9.16 ± 0.01 mg/g) and ellagic acid (9.03 ± 0.02 mg/g) were detected as major compounds into CPF-246 sugarcane leaves extract. The results of the study showed that these cultivars might be used not only as an accessible source of natural antioxidants but also as an ingredient in functional foods (Abbas SR, Sabir SM, Ahmad SD, Boligon AA, Athayde ML. Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). *Food Chem* 2014; 147: 10-16)

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