CHAPTER V

SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PINDOLOL IN PURE FORM AND PHARMACEUTICAL FORMULATIONS
SECTION-5.1

**Brief introduction to Pindolol:**

Indole and its derivatives represent one of the most active classes of organic compounds possessing a wide spectrum of biological activity. Indole is present in Jasmine flower which has a pleasant fragrance. It is found both in natural and synthetic perfumes. It is used in the manufacture of tryptophan, indole 3-acetic acid, synthetic halogenated indigo dyes for cellulose fabric etc. Indole ring is found in a large group of natural products obtained from both plant and animal kingdoms. It is also an equally important nucleus in many drugs that have therapeutic value and Pindolol is one of them which is widely used for treatment of hypertension.

Pindolol, 1-(1H-indole-4-yloxy)-3-(1-methylethylamino) propan-2-ol, (PDL) is a potent non-subtype-selective beta blocker [1]. It also shows membrane-stabilizing effects like quinidine, possibly accounting for its anti-arrhythmic effects [2]. Its molecular weight is 405.49.

This drug is quite often researched as an adjunct in the treatment of depression. The drug is commonly used in the management of Hypertension [3] and Angina pectoris [4].
Comprehensive review of the literature:

A survey of literature revealed that there are only few chromogenic reagents reported for the estimation of PDL using spectrophotometry so far. Though the chromatographic methods are sensitive, they require intricate procedures. Some of these methods are time consuming and suffer from lack of good sensitivity. This drug is included in British Pharmacopoeia where it is analyzed by potentiometric method [5]. Few other methods have been reported for the estimation of PDL which include HPLC [6-7], GC [8-9], UV-visible spectrophotometry [10-15], derivative spectrophotometry [16], spectrofluorometry [17] etc.

Mahrous et al [10] described two spectrophotometric methods based on the oxidation of indole moiety of Pindolol using potassium persulphate and hydrogen peroxide with absorption maxima of 535 nm and 570 nm respectively. Beer’s law is obeyed over the range 7-35 μg ml⁻¹ for potassium persulphate and 10-50 μg ml⁻¹ for hydrogen peroxide.

Pecanac et al. [11] developed a method by which Pindolol reacts with Fe(III) chloride producing a green water soluble complex (1:1, v/v) having maximum absorbance at 635 nm. Beer’s law was obeyed upto a
concentration of 220 μg ml\(^{-1}\). This method depends mainly on pH of the reaction medium.

**Sastry et al.** [12] developed a method for the determination of Pindolol, based upon the oxidation of the drug by sodium nitrite in acidic condition and coupling of oxidized Pindolol under acidic conditions and its coupling of the oxidised intermediate with N-1-(naphthyl) ethylenediamine dihydrochloride to generate a coloured product, having maximum absorbance at 660 nm. The author also proposed other couplers for the same oxidized Pindolol, such as diphenylamine or 1-naphthylamine which also react to give coloured products with same absorption maxima in both the cases.

**Zakhari, et al.** [13] proposed a spectrophotometric method for the estimation of Pindolol. They used diazotized 4-nitroanalinine as a chromogenic reagent for the determination of Pindolol.

**Issa et al.** [14] reported a method for the quantification of Pindolol using 2,3-dichloro-5,6-dicyano-p-benzoquinone as a \(\pi\)-acceptor. The highly coloured anion radical had \(\lambda_{\text{max}}\) of 460nm and Beer’s law range was obeyed between 8 to 40 μg ml\(^{-1}\).  

123
Sastry et al. [15] again proposed a method for determination of Pindolol which involved the addition of excess of sodium nitrite to the compound in the presence of 0.25 M hydrochloric acid solution and the unreacted nitrous acid is determined by the measurement of corresponding decrease in the absorbance of cresyl fast violet acetate having $\lambda_{\text{max}}$ at 555 nm.

In the present study, the investigator has succeeded in developing a spectrophotometric method for the sensitive and selective determination of Pindolol in which the drug get oxidised to derivatives in the presence of sodium nitroprusside. The indoxyl derivative undergoes dimerization to Indigo in the presence of phosphoric acid.
SECTION 5.2

Instrument and reagents:
Experimental

Apparatus:

A CHEMITO MAKE UV-VIS Spectrophotometer (MODEL No. 2100) with 1.0 cm matched cells was used for all spectral and absorbance measurements.

Reagents:

Pharmaceutical grade PDL was obtained as gift sample from Novartis Pharmaceuticals, Canada Inc., Canada. Sodium nitroprusside was of Merck make and Phosphoric acid of Ranbaxy. All other chemicals used were of analytical reagent grade.

Deionized water & absolute alcohol was used throughout the experiment. Commercial dosage forms were purchased from local sources.

Preparation of standard PDL Solution:

Accurately weighed PDL (100mg) was transferred into a 100ml standard flask dissolved in alcohol and made up to the volume with water. The working standard solutions of PDL were prepared by further dilutions.
Preparation of reagents and chemicals:

All the chemicals and reagents used were of AR grade. Deionized water was used to prepare all the solutions and in all experiments.

1. Sodium nitroprusside (0.002M) : Prepared by dissolving 52mg of Sodium nitroprusside (SNP) in 100 ml. of water.

2. Sodium hydroxide (1 M) : Prepared by dissolving 4.0g of Sodium hydroxide in 100ml water.

3. Phosphoric acid (1:1) : Prepared by diluting 50ml of Phosphoric acid to 50ml with water.
SECTION 5.3

DETERMINATION OF PINDOLOL

General procedure:

Aliquots of working standard solution of reduced PDL (5-180 µg) were transferred into a series of 10ml calibrated flasks and to each, 1 ml of solution of Sodium nitroprusside (0.002 M) was added. Then 1.0 ml solution of Sodium hydroxide (1M) was added to each flask followed by 1.0 ml of solution of Phosphoric acid (1:1). The resulting coloured product was made up to mark with water. The solutions were allowed to stand for 15 minutes and absorbance was measured at 575 nm. Calibrated graph was constructed by plotting concentration against absorbance to get the range of determination for the proposed method. Fig. 5.3.1 shows Beer’s law plot for PDL in the adjoining page.
Procedure for the assay of PDL in pharmaceutical preparations:

**Tablets:**

To estimate PDL in pharmaceutical formulations for tablets, the investigator took twenty tablets, weighed then, powdered and mixed thoroughly. An amount equivalent to 100mg of PDL was taken, dissolved in alcohol and filtered. The filtrate was made up to 100ml with water and aliquot of this solution was treated as described above for the determination of PDL as in general procedure.
Results and Discussion

Absorption spectra:

The proposed methods involved the formation of a blue coloured product, when PDL was oxidized in alkaline medium using sodium nitroprusside and acidified with phosphoric acid product. In order to determine the absorption maximum for PDL, a specified amount of PDL (which was half of the Beer’s law range) was taken and the coloured reaction products were developed separately as mentioned in general procedure.

The absorption spectra were scanned in UV-visible region between 400 and 800 nm. The absorption maximum for blue coloured reaction product was found to be 575 nm. Fig. 5.3.2 shows absorption spectrum for PDL in the adjoining page.
CHAPTER-V
SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF
PINDOLOL IN PURE FORM AND PHARMACEUTICAL
FORMULATIONS

Fig. 5.3.2 Absorption spectra of PDL.

Optimization of experimental parameters:

Various concentrations and volume ranges for all reagents used in the proposed methods were studied in details by systematically carrying out the investigations. The absorbance of a series of solutions was measured by varying one and fixing the other parameters in each case to achieve the necessary conditions for the formation of stable colour intensities. The recommended volumes for all the reagents is mentioned in general procedure.
**Effect of solvents:**

The effect of acids on the stability and the sensitivity of the reaction product were checked by using different concentrations of sulfuric acid, hydrochloric acid and phosphoric acid. Nitric acid could not be used because of its strong oxidizing capacity. It was found that 1:1 solution of phosphoric acid gave the maximum colour intensity and stability for the reaction products.

**Stability:**

A stable colour was developed when the reaction mixture was allowed to stand for 10 min, which remained unaltered for minimum period of 5hrs. The stability of the coloured product was also studied with reference to temperature. The results indicated that the absorbance values remained constant upto 100°C.

**Interference:**

Under the reaction conditions, the problem of interference of these compounds did not arise in analysis of commercially available PDL dosage forms. The effect of additives associated with PDL in its formulations was investigated using developed methods. These methods do not suffer any interference from common excipients and additives such as magnesium.
stearate, glucose, dextrose, lactose, starch, gum acacia, carboxy methyl cellulose and sodium alginate.

The results are given in Table 5.3.1.

**Table [5.3.1]: Determination of PDL * in the presence of excipients.**

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Amount of excipients added (mg)</th>
<th>% Recovery of PDL*, ± % RSD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum acacia</td>
<td>100</td>
<td>99.8 ± 0.8</td>
</tr>
<tr>
<td>Talc</td>
<td>150</td>
<td>99.8 ± 0.7</td>
</tr>
<tr>
<td>Starch</td>
<td>100</td>
<td>99.5 ± 0.6</td>
</tr>
<tr>
<td>Dextrose</td>
<td>100</td>
<td>99.5 ± 0.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>200</td>
<td>99.7 ± 0.7</td>
</tr>
<tr>
<td>Lactose</td>
<td>50</td>
<td>99.5 ± 0.7</td>
</tr>
<tr>
<td>Carboxy methyl cellulose</td>
<td>100</td>
<td>99.7 ± 0.6</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>50</td>
<td>100.1 ± 0.8</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>100</td>
<td>99.6 ± 0.7</td>
</tr>
</tbody>
</table>

* 9.0 μg ml⁻¹ of PDL taken for optical measurements.

**Average of six replicate determinations.
Quantification:

Adherence to Beer's law by the coloured species formed in this method was tested by measuring the absorbance of a set of solutions containing varying amounts of PDL and specified amounts of reagents (as given in general procedure) against the corresponding reagent blank at appropriate wavelengths. Limit of quantification (LOQ) is determined by taking the ratio of standard deviation ($\sigma$) of the blank with respect to water and the slope of the calibration curve ($s$) multiplied by a factor 10. Limit of detection (LOD) is determined by taking the ratio of standard deviation ($\sigma$) of the blank with respect to water and the slope of the calibration curve ($s$) multiplied by a factor 3.3. The upper limit of the Beer-Lambert's range is determined by series of concentrated solutions and lower limit of Beer-Lambert's range was determined by a series of diluted solutions at the value of $\lambda_{max}$. Beyond this limit, the correlation results were really affected. Hence, the measurements were excluded above and below these limits to keep the relationship linear. The optical parameters such as Beer's law range, molar absorptivity, Sandell's sensitivity, optimum photometric range, LOD, LOQ, correlation coefficient, slope and Intercept were calculated using the least square regression analysis and are recorded in Table 5.3.2.
Table [5.3.2]: Parameters for the Spectrophotometric determination of PDL

<table>
<thead>
<tr>
<th>Parameter/characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Blue</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>575</td>
</tr>
<tr>
<td>Stability (hours)</td>
<td>5</td>
</tr>
<tr>
<td>Beer’s law range (µg ml$^{-1}$)</td>
<td>0.5-18</td>
</tr>
<tr>
<td>Limit of detection (µg ml$^{-1}$)</td>
<td>0.1297</td>
</tr>
<tr>
<td>Limit of quantitation(µg ml-1)</td>
<td>0.432</td>
</tr>
<tr>
<td>Molar absorptivity (l mol-1 cm-1)</td>
<td>9.0 X 10³</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg cm-2)</td>
<td>0.0275</td>
</tr>
<tr>
<td>Optimum photometric range(µg ml$^{-1}$)</td>
<td>1-17</td>
</tr>
<tr>
<td>Regression equation (Y$*$)</td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.0393</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>-0.0193</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9937</td>
</tr>
<tr>
<td>Relative standard deviation (%)</td>
<td>0.255</td>
</tr>
<tr>
<td>Range of error (at 95 % confidence level)</td>
<td>± 0.353</td>
</tr>
</tbody>
</table>

*Y=a x + b where x is the concentration in µg ml$^{-1}$
Application:

The reproducibility of the method was checked by conducting six replicate determinations at 9.0 μg mL⁻¹ PDL and the standard deviation was found to be between 0.20 and 1.2 %. [5.3.3]. These results were highly reproducible. The results of assay of tablets were cross checked by comparison with the official method.

Table [5.3.3]: Determination of PDL in Pharmaceutical dosage forms.

<table>
<thead>
<tr>
<th>Commercial formulations analyzed</th>
<th>Label claim (mg)</th>
<th>Amount of drug found* in mg</th>
<th>t-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proposed method</td>
<td>Official method (b)</td>
<td></td>
</tr>
<tr>
<td>Visken(a)</td>
<td>5</td>
<td>4.9 ± 0.2</td>
<td>4.95 ± 0.34</td>
<td>1.025</td>
</tr>
<tr>
<td>Visken(a)</td>
<td>10</td>
<td>9.73 ± 0.20</td>
<td>9.6 ± 0.45</td>
<td>1.020</td>
</tr>
</tbody>
</table>

* Average of six determinations ± % RSD

Theoretical t-value = 2.776
Theoretical F-value = 6.39

Precision and accuracy of the proposed method:

The precision of the proposed method was checked by carrying out six replicate determinations of specified amounts of PDL (within Beer’s law
Low values of RSD (%) and range of error determine the precision of the proposed methods. Table 5.3.4 shows paired t-Test data to substantiate the accuracy of the method.

**Table 5.3.4: Paired t-test data for PDL**

<table>
<thead>
<tr>
<th>Concentration of PDL taken in ( \mu g \text{ ml}^{-1} )</th>
<th>Concentration found * in mg</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Official Method</td>
<td>Proposed Method</td>
</tr>
<tr>
<td>0.5</td>
<td>0.502</td>
<td>0.499</td>
</tr>
<tr>
<td>1.0</td>
<td>0.998</td>
<td>1.001</td>
</tr>
<tr>
<td>2.0</td>
<td>1.985</td>
<td>2.001</td>
</tr>
<tr>
<td>4.0</td>
<td>4.008</td>
<td>4.001</td>
</tr>
<tr>
<td>8.0</td>
<td>7.998</td>
<td>8.001</td>
</tr>
<tr>
<td>16</td>
<td>16.002</td>
<td>16.995</td>
</tr>
<tr>
<td>18</td>
<td>17.825</td>
<td>17.985</td>
</tr>
</tbody>
</table>

*Average of six determinations

Theoretical t-Value = 2.776

**Proposed Reaction mechanism:**

Pindolol gets oxidized to indoxyl derivative in the presence of sodium nitroprusside. The indoxyl derivative then undergoes dimerization to Indigo in the presence of Phosphoric acid. It was found that freshly prepared SNP did not maximize colour intensity. Hence, a day old SNP solution was necessary to achieve maximum colour intensity at room temperature.
CHAPTER-V

SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PINDOLOL IN PURE FORM AND PHARMACEUTICAL FORMULATIONS

Conclusion:

The present work in this chapter highlights the importance of the determination of PDL by the proposed method in pure form and pharmaceutical preparations. The proposed method is simple, rapid, accurate and highly sensitive than most of the spectrophotometric methods currently available in the literature. The sensitivity, simplicity, temperature independence and stability of the coloured product are the advantages of this
method and it does not involve extraction step and use of carcinogenic solvents. The proposed method does not entail any stringent experimental variables which affect the reliability of the results. From the recovery studies it is evident that this method can serve as an alternative method for determining Pindolol in pharmaceutical dosage forms.

The statistical and optical characteristic data clearly indicate the reproducibility and accuracy of the method. The general procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high degree of its quality control.
REFERENCES:


CHAPTER-V
SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF
PINDOLOL IN PURE FORM AND PHARMACEUTICAL
FORMULATIONS


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