Spectrophotometric Assay of Phenylephrine hydrochloride in Pharmaceutical Formulation with Alizarin Red sulphonate in Aqueous Solution

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ABSTRACT
A simple, rapid and sensitive spectrophotometric method for the determination of phenylephrine hydrochloride was developed. The method is based on the proton transfer reaction with alizarin red sulphonate in aqueous neutral solution to form a violet product showing a maximum absorbance at 530 nm with molar absorptivity of 14320 l.mol\(^{-1}\).cm\(^{-1}\). The method is obeyed Beer’s law over the concentration range (0.5-10) µg.ml\(^{-1}\). The accuracy (average recovery) of the method is 99.72% and the precision (RSD) of the method is less than 1.5%.

The method was successfully applied for the determination of phenylephrine hydrochloride in pharmaceutical formulation as eye drops and the results were in good agreement with the standard addition procedure.

INTRODUCTION
Phenylphrine(pp) is a decongestant has the following chemical structure\(^{(1)}\).
It works by constricting blood vessels (veins and arteries). Constriction of blood vessels in the sinus, nose, and chest allows drainage of these areas, which decreases congestion. Constriction of blood vessels also affects blood pressure. When taken by mouth, PP is used to treat congestion associated with allergies, hay fever, sinus irritation, and the common cold. When used by injection, PP is used to maintain adequate blood pressure and to treat certain types of irregular heartbeats (2-4).

A spectrophotometric determination of PP in pharmaceutical preparations via oxidative coupling reaction with 4-aminoantipyrine in the presence of sodium periodate, the absorbance of colored compound was measured at 500 nm (5).

Spectrophotometric determination of PP hydrochloride in pharmaceuticals by flow injection analysis exploiting the reaction with potassium ferricyanide and 4-aminoantipyrine was developed. The red product shows maximum absorption at 500 nm (6). n-π CT-complex formation reaction has been used for the determination of PP spectrophotometrically with picric acid and m-dinitrobenzene as π acceptors (7). A new spectrophotometric method is proposed for determination of PP. The method is based on the coupling of 4-aminoantipyrine with PP to give a new ligand that reacts with copper (II) at pH 9 to give an intense red colored chelate at 480 nm (8). PP has been determined by high performance liquid chromatography in human serum using column switching with fluorescence detection (9), colorimetric detection (10), in capsules with UV detection at 215 nm (11).

In this work alizarin red sulphonate reagent was used for the spectrophotometric determination of phenylphrine via proton transfer reaction in aqueous solution.

**EXPERIMENTAL**

**Apparatus**

A Shimadzu UV-210 A digital double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurements.

**Reagents**

PP hydrochloride and alizarin red sulphonate (ARS) used were produced by BDH company. All other chemicals used were of analytical-reagent grade. Distilled water was used for the preparations of all solutions except...
ARS solution was prepared in absolute ethanol, pp hydrochloride was dissolved in 5 ml of ethanol then completed with distilled water.

Solutions
pp hydrochloride: 100 μg.ml⁻¹ of the pure drug was freshly prepared by dissolving 0.01 g in 100 ml distilled water. This solution is protected from sun light and used as a standard solution for the analytical purposes. ARS: 1 × 10⁻³ M was prepared by dissolving 0.0856 gm in 250 ml of absolute ethanol. This solution was kept in brown bottle and was stable for, at least, one month.

Procedure
To a series of 10 ml calibrated flasks, transfer an increasing volumes of pp hydrochloride working standard solution (100 μg.ml⁻¹ ) to cover the range (0.5–10) μg.ml⁻¹ in final dilution. Add 2 ml of 1 × 10⁻³ M ARS. Dilute the solution to the mark with distilled water, stopper the flasks and shake well, then allow the reaction mixtures to stand for 5 min. at room temperature. The absorbance is measured at 530 nm against the reagent blank, using 1 cm cells.

Assay procedure for phenylephrine hydrochloride drops:
Three droppers of pp hydrochloride (50 mg) marked by Sina Darou Tehran-Iran were mixed well then an equivalent volume to half dropper was taken and diluted with distilled water and completed to the mark in a 250 ml volumetric flask, then different volumes used from this solution containing the concentration range (0.5–10) μg.ml⁻¹. After that, following the procedure described above.

RESULTS AND DISCUSSION

Absorption spectra
pp hydrochloride was reacted with ARS to produce a violet coloured proton transfer product with a maximum absorption at 530 nm while the reagent blank shows low absorption at this wavelength (Fig. 1).

Optimization of conditions
Effect of buffer solution
The pH of the pure drug solution was 8.0. And the final pH after the addition of 2 ml of ARS was 7.0, therefore different buffers of pH 7.0 were prepared such as borate, carbonate, acetate and phosphate. It was found that these buffers show decrease in the color intensity therefore the reaction was carried on a neutral medium (Table 1).

<table>
<thead>
<tr>
<th>Buffer solutions (pH 7.0)</th>
<th>Borate</th>
<th>Carbonate</th>
<th>Acetate</th>
<th>Phosphate</th>
<th>Without</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.021</td>
<td>0.007</td>
<td>0.030</td>
<td>0.024</td>
<td>0.321</td>
</tr>
</tbody>
</table>
Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath adjusted at 0, 40 and 50 °C. The absorbance was measured at 10 mins. intervals against reagent blank treated similarly. It was observed that the colored product absorbance reached maximum after 5 mins. at room temperature and remained constant for 115 mins., whereas the absorbance decreased slowly thereafter. Hence room temperature and reaction time 5 mins., were chosen for color development (Table 2).
Spectrophotometric Assay of Phenylephrine hydrochloride in ....

Table (2): Effect of temperature and reaction time

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Absorbance Time (min)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>90</th>
<th>100</th>
<th>120</th>
<th>130</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>0.315</td>
<td>0.316</td>
<td>0.316</td>
<td>0.317</td>
<td>0.320</td>
<td>0.315</td>
<td>0.315</td>
<td>0.314</td>
<td>0.312</td>
<td>0.314</td>
<td>0.314</td>
</tr>
<tr>
<td>RT</td>
<td></td>
<td>0.350</td>
<td>0.318</td>
<td>0.350</td>
<td>0.349</td>
<td>0.350</td>
<td>0.348</td>
<td>0.350</td>
<td>0.350</td>
<td>0.350</td>
<td>0.345</td>
<td>0.345</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>0.318</td>
<td>0.318</td>
<td>0.318</td>
<td>0.320</td>
<td>0.320</td>
<td>0.322</td>
<td>0.325</td>
<td>0.330</td>
<td>0.330</td>
<td>0.325</td>
<td>0.325</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>0.318</td>
<td>0.318</td>
<td>0.322</td>
<td>0.326</td>
<td>0.327</td>
<td>0.333</td>
<td>0.332</td>
<td>0.330</td>
<td>0.330</td>
<td>0.325</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Effect of ARS reagent concentration

The effect of different ARS concentrations on the absorbance of colored solution containing 4 μg.ml⁻¹ pp hydrochloride was studied, 2 ml of 1 × 10⁻³ M ARS, give the maximum absorbance of the reaction product, therefore, this concentration was used in all subsequent work (Table 3).

Table (3): Effect of ARS concentration

<table>
<thead>
<tr>
<th>ARS solution 1×10⁻³ M (ml)</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.285</td>
<td>0.298</td>
<td>0.321</td>
<td>0.293</td>
<td>0.290</td>
</tr>
</tbody>
</table>

Effect of surfactant

The effect of different types of surfactants were used for the improvement of the absorption intensity by two orders of reagents addition. The results shown in Table (4) confirm that there is no improvement in the color intensity of the product, therefore they were excluded.

Table (4): Effect of surfactant

<table>
<thead>
<tr>
<th>Surfactant 0.1%</th>
<th>Absorbance / ml</th>
<th>Order</th>
<th>Without</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80*</td>
<td></td>
<td></td>
<td></td>
<td>0.320</td>
<td>0.318</td>
<td>0.319</td>
<td>0.319</td>
</tr>
<tr>
<td>CPC</td>
<td></td>
<td>0.321</td>
<td></td>
<td>0.317</td>
<td>0.318</td>
<td>0.317</td>
<td>0.317</td>
</tr>
<tr>
<td>SDS</td>
<td></td>
<td></td>
<td></td>
<td>0.319</td>
<td>0.316</td>
<td>0.316</td>
<td>0.317</td>
</tr>
</tbody>
</table>

* 1%
I pp + ARS + surfactant
II pp + surfactant + ARS

Analytical data

Under the optimized experimental conditions, a linear relation between the absorbance and the concentration of pp hydrochloride was observed cover the concentration range (0.5-10) μg.ml⁻¹ (Fig. 2) with correlation coefficient of 0.9998 and intercept of 0.0386. A negative deviation from Beer’s law was observed at higher concentrations of pp hydrochloride. The molar absorptivity was 14320 l.mol⁻¹.cm⁻¹.
Figure (2): Calibration graph of pp hydrochloride

Accuracy and precision
To determine the accuracy and precision of the method, pp hydrochloride was determined at three different concentrations of six replicates. The results shown in table (5) referring a satisfactory precision and accuracy.

Table (5): Accuracy and precision of the proposed method

<table>
<thead>
<tr>
<th>Amount of pp hydrochloride taken µg.ml⁻¹</th>
<th>Recovery* %</th>
<th>Average recovery %</th>
<th>Relative standard deviation* (RSD%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>99.07</td>
<td>99.72%</td>
<td>1.13</td>
</tr>
<tr>
<td>5</td>
<td>100.76</td>
<td>99.72%</td>
<td>0.61</td>
</tr>
<tr>
<td>6</td>
<td>99.35</td>
<td>99.72%</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* Average for six determinations

Nature of product and reaction mechanism
The stiochiometry of the reaction between the drug and ARS was investigated using job's method\(^{(12)}\), with using equimolar solutions of each (1x10⁻³ M). It was found that PP forms a product with ARS in the ratio of 1:1 as it is evident in figure 3.

Figure (3): Job’s method for pp hydrochloride-ARS product
The formation of the product may be occur as follows\(^{(13)}\):

\[
\begin{align*}
\text{O} & \quad \text{OH} & \quad \text{OH} & \quad \text{SO}_3\text{Na} \\
\text{O} & \quad \text{OH} & \quad \text{HO} & \quad \text{CH} - \text{CH}_2\text{NH}_2\text{Me} \cdot \text{Cl}
\end{align*}
\]

The stability constant of the product was estimated and found to be \(3.71 \times 10^5 \text{ l.mol}^{-1}\).

**Analytical application**

The proposed method was applied to the determination of pp hydrochloride in pharmaceutical eye drops. Good recovery was obtained and the results were compared with the standard addition method (Fig. 4 and Table 6).

![Figure (4): Standard addition graphs of pp hydrochloride in pharmaceutical formulation](image)

**Figure (4): Standard addition graphs of pp hydrochloride in pharmaceutical formulation**
Table (6): Assay of pp hydrochloride in eye drops

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Certified value (mg)</th>
<th>Drug content (mg) found by</th>
<th>Recovery (%) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct ARS method</td>
<td>Standard addition method</td>
</tr>
<tr>
<td>Phenylephrine hydrochloride drops 0.5%</td>
<td>50</td>
<td>49.68</td>
<td>50.50</td>
</tr>
</tbody>
</table>

*Average of three determinations

Comparison of the method with other methods

Table (7) gives the results obtained by application of proposed method and literature method for the determination of pp hydrochloride in pharmaceutical preparations.

Table (7): Comparison of the present method with other method

<table>
<thead>
<tr>
<th>Analytical parameter</th>
<th>Present method</th>
<th>3.5-DNS method(^{(14)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>530</td>
<td>410</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td>R.T</td>
<td>R.T</td>
</tr>
<tr>
<td>pH</td>
<td>Without</td>
<td>Basic medium</td>
</tr>
<tr>
<td>Development time (min)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Stability period (min)</td>
<td>115</td>
<td>90</td>
</tr>
<tr>
<td>Molar absorptivity (\text{L.mol}^{-1}.\text{cm}^{-1})</td>
<td>14320</td>
<td>8660</td>
</tr>
<tr>
<td>Linearity ((\mu\text{g.ml}^{-1}))</td>
<td>0.5-10</td>
<td>0.5-20</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>99.72</td>
<td>99.80</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>&lt; 1.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Analytical application</td>
<td>eye drops</td>
<td>eye drops</td>
</tr>
</tbody>
</table>

From the results shown in table (7) there is similarity in recovery, both methods done in room temperature in addition the present method is more sensitive, without buffer solution, simple and does not require solvent extraction.

CONCLUSION

A simple, precise and sensitive spectrophotometric method has been developed for the determination of pp based on proton transfer reaction between pp and ARS reagent to form colored product. The method doesn’t involve the use of complicated sample preparation or buffer solutions. Low value of standard deviation shows that the method is precise, whereas high percentage of recovery shows that the method is free from interference of the excipients used in the formulations. The method was successfully applied for the determination of pp in pharmaceutical formulation as eye drops and the results were in good agreement with the standard addition procedure.
REFERENCES