Spectrophotometric estimation of tamsulosin hydrochloride by acid-dye method

A new spectrophotometric method for the estimation of tamsulosin hydrochloride in pharmaceutical dosage forms has been developed and validated. The method is based on reaction between drug and bromophenol blue and complex was measured at 421 nm. The slope, intercept and correlation coefficient was found to be 0.054, -0.020 and 0.999, respectively. Method was validated in terms of specificity, linearity, range, precision and accuracy. The developed method can be used to determine drug in both tablet and capsule formulations. Reaction was optimized using three parameters i.e., concentration of the dye, pH of the buffer, volume of the buffer and shaking time. Maximum stability of the chromophore was achieved by using pH 2 and 2 ml volume of buffer. Shaking time kept was 2 min and concentration of the dye used was 2 ml of 0.05% w/v solution. Method was validated in terms of linearity, precision, range, accuracy, LOD and LOQ and stochiometry of the method was also established using Mole ratio and Job’s method of continuous variation. The dye benzoid form (blue color) of dye ionized into quinoid form (purple color) in presence of buffer and reacts with protonated form of drug in 1:1 ratio and forms an ion-pair complex (yellow color).

**Key words:** Bromophenol blue, method development and validation, spectrophotometric estimation tamsulosin hydrochloride

INTRODUCTION

Tamsulosin hydrochloride 5-[(2R)-2-[2-(2-ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzenesulfonamide hydrochloride [Figure 1] is a uroselective $\alpha_{1A}$ ($\alpha_{1A}$, $\alpha_{1B}$ affinity 7-38-fold) antagonist which is used in benign prostatic hyperplasia (BPH). The $\alpha_{1A}$-receptors are prominent in prostate, prostatic capsule, prostatic urethra and bladder where it acts by relaxation of prostate and bladder smooth muscles helps to urine flow, reduction of lower urinary tract symptoms and decrease urinary hesitancy/urgency. The medication is available in single or in combination with dutasteride or finasteride.[1] Tamsulosin is official in European pharmacopoeia.[2]

available for the estimation by UV spectroscopy which is far simpler, economical and less time consuming as compared to above-mentioned methods.

The acid-dye method can provide a more sensitive technique for certain amines and quaternary ammonium compounds that absorb weakly in the ultraviolet region. In such methods addition of an amine in its ionized form to an ionized acidic dye, yields a salt (ion-pair) that may be extracted into an organic solvent such as chloroform or dichloromethane. The indicator dye is added in excess and the pH of the aqueous solution is adjusted (if necessary) to a value where both the amine and dye are in ionized forms. The ion-pair is separated from the excess indicator by extraction into the organic solvent, and the absorbance is measured at the $\lambda_{\max}$ of the indicator in the solvent.\[21\] TAM exist as secondary ammonium salt, thus acid-dye method is found suitable for increasing the sensitivity of the drug. Hence this forms sufficient basis for the development of such type of method for Tamsulosin also. Further validation of the proposed method was planned to be performed as per ICH guidelines\[22\].

MATERIALS AND METHODS

Pure tamsulosin hydrochloride was received as gift sample by Aurobindo Pharma Ltd., Hyderabad, India. UV-Visible spectrophotometer of Shimadzu Corporation model UV-1800 was used in the estimation. Methanol, bromophenol blue, potassium chloride, concentrated HCl and chloroform were purchased from Loba Chemie Pvt. Ltd. and were of GR grade.

Preparation of reagents and solutions

**Dye solution**

0.05% w/v dye solution was freshly prepared by dissolving the dye in distilled water.

**HCl-KCl buffer**

Buffer was prepared according to I.P. method by mixing 0.2 M KCl and a suitable amount of 0.2 M HCl to obtain the buffer of required pH.

**Standard solution of drug**

Standard stock of drug was prepared by dissolving 50 mg of pure drug in methanol and diluted 10 ml to obtain a standard solution of 5000 $\mu$g/ml. 2.5 ml of this stock was diluted 50 ml to obtain a working standard of 250 $\mu$g/ml.

Optimization of the reaction conditions

Reaction was optimized using three parameters i.e., concentration of the dye, pH of the buffer, volume of the buffer and shaking time. Maximum stability of the chromophore was achieved by using pH 2 and 2 ml volume of buffer. Shaking time kept was 2 min and concentration of the dye used was 2 mL of 0.05% w/v solution. Figure 2 clearly indicate the increase in absorbance of TAM after reaction with dye.

**Choice of concentration of dye**

From the literature it was revealed that in acid dye complexation method the amount of dye should be in excess. The ion-pair between the drug and dye formed is in 1:1 ratio. Thus, 2 ml of 0.05% w/v solution of dye will be sufficient for the proposed method.

**Shaking time**

As the drug was soluble in methanol and dye in water, so ion-pair was formed in aqueous layer. Therefore, the shaking time should be sufficient enough to extract the ion-pair of drug and dye from the aqueous layer to organic layer and 2 min shaking time was selected for extraction.

**Volume and pH of buffer**

HCl-KCl buffer was selected for the purpose, different pH and volume was used to optimize this parameter. The condition showing maximum absorbance and stability is the basis of selection of optimized condition. This is obvious from the results

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**Figure 1:** Chemical structure of tamsulosin hydrochloride

**Figure 2:** Overlay spectra of pure TAM (a) 200 $\mu$g/ml in methanol and (b) Ion-pair complex (10 $\mu$g/ml with BPB in chloroform)
obtained after optimizing reaction that the maximum absorbance and stability conditions of the complex is attained at pH 2 and volume 2 ml of buffer. The summary of optimization studies and stability of product after reaction is presented under Table 1 and Figure 3, respectively.

Preparation of calibration curve for TAM
In a series of separating funnel, aliquots of standard drug solution (250 µg/ml) of TAM (0.1, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.9 ml) were transferred, 2 ml of buffer (pH 2.0) was added for ionization and 2 ml of dye solution was added, 10 ml of chloroform was transferred to each separating funnel, shaken for 2 min and allowed to stand for 5 min for complete separation of aqueous and organic layers and yellow-colored ion-pair complex in organic layer was extracted and final volume was made up to 10 ml with chloroform in 10 ml volumetric flask to obtain 2.5, 7.5, 10, 12.5, 15, 17.5 and 22.5 µg/ml concentration. Same procedure was repeated two times in a day for 3 days. Calibration curve was plotted [Figure 4] by using mean absorbance of these 3 days.

VALIDATION

Specificity
Excipients like carboxy methyl cellulose, talc, starch and magnesium stearate were mixed in proportion approximately 80, 8, 15 and 4 mg, respectively. They were mixed with 250µg/ml stock in a 25-ml volumetric flask, mixed and diluted up to the mark. Interference by these excipients was found to be 0.375% (<0.5%) proves specificity of the method.

Linearity

Visualizing method
Out of seven concentration levels in the calibration curve [Figure 4] three points lies above, three below and one on the calibration line shows the linearity by visualizing the graph.

Plot of residuals
Residuals were found to be distributed between upper and lower side of the line when plotted against concentration.[23] Linearity was further assessed by Dixon's test proves no outlier in the calibration curve. [24] Table 2 and Figure 5 is graph of residuals plotted against concentration. Dixon test of Outliers:

Result: There are no outliers in the data of calibration curve according to Dixon test.

Ascending series of data of calibration curve and Data of Dixon test for outliers are presented under Table 2 and 3 respectively.
**Linear function analysis:** Linear function analysis or lack of fitness test is applied by calculation of $SS_r$, $SS_e$, $SS_{lof}$ and their respective variances. The applicability of the method was analyzed by comparing the tabulated and calculated F ratio [Table 3].

Data of residual error sum squares and pure error sum squares are presented under Tables 4 and 5 respectively.

Calculation of error sum of squares: [Tables 4 and 5]

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**Table 2: Ascending series of data of calibration curve**

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>2.5</td>
<td>0.115</td>
<td>0.12</td>
<td>0.122</td>
<td>0.125</td>
<td>0.126</td>
<td>0.129</td>
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<td>7.5</td>
<td>0.365</td>
<td>0.371</td>
<td>0.374</td>
<td>0.379</td>
<td>0.385</td>
<td>0.393</td>
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<tr>
<td>10</td>
<td>0.518</td>
<td>0.526</td>
<td>0.529</td>
<td>0.531</td>
<td>0.536</td>
<td>0.542</td>
</tr>
<tr>
<td>12.5</td>
<td>0.624</td>
<td>0.644</td>
<td>0.649</td>
<td>0.659</td>
<td>0.665</td>
<td>0.669</td>
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<tr>
<td>15</td>
<td>0.769</td>
<td>0.779</td>
<td>0.781</td>
<td>0.789</td>
<td>0.795</td>
<td>0.801</td>
</tr>
<tr>
<td>17.5</td>
<td>0.919</td>
<td>0.926</td>
<td>0.933</td>
<td>0.936</td>
<td>0.939</td>
<td>0.947</td>
</tr>
<tr>
<td>22.5</td>
<td>1.187</td>
<td>1.195</td>
<td>1.206</td>
<td>1.216</td>
<td>1.219</td>
<td>1.221</td>
</tr>
</tbody>
</table>

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**Table 3: Data of Dixon test for outliers**

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>$r$ smallest</th>
<th>$r$ largest</th>
</tr>
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<tbody>
<tr>
<td>2.5</td>
<td>0.3571</td>
<td>0.21429</td>
</tr>
<tr>
<td>7.5</td>
<td>0.2143</td>
<td>0.28571</td>
</tr>
<tr>
<td>10</td>
<td>0.3333</td>
<td>0.08889</td>
</tr>
<tr>
<td>12.5</td>
<td>0.3125</td>
<td>0.1875</td>
</tr>
<tr>
<td>15</td>
<td>0.25</td>
<td>0.28571</td>
</tr>
<tr>
<td>17.5</td>
<td>0.2353</td>
<td>0.05882</td>
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</table>

Limit at 5% significance level: 0.560

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**Table 4: Data of residual error sum squares**

<table>
<thead>
<tr>
<th>X</th>
<th>$(y_i - \bar{y_i})^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>3.025E-05</td>
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<tr>
<td>7.5</td>
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<td>22.5</td>
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</tr>
</tbody>
</table>

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**Table 5: Data of pure error sum squares**

<table>
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</thead>
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<tr>
<td>7.5</td>
<td>1.46944E-05</td>
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<td>10</td>
<td>1.77778E-06</td>
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<td>12.5</td>
<td>1E-04</td>
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<td>0.000261361</td>
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<tr>
<td>17.5</td>
<td>0.000205444</td>
</tr>
<tr>
<td>22.5</td>
<td>0.000427111</td>
</tr>
</tbody>
</table>

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Figure 6: Overlay spectra of TAM (2.5, 7.5, 10, 12.5, 15, 17.5 and 22.5 µg/ml) by the proposed method.
Powdered veltam tablets equivalent to 6.25-mg TAM was transferred to 25-ml volumetric flask and ultrasonication was done for 10 minutes with approximately 20-ml methanol. Solution was then diluted up to the mark with methanol and filtered through 0.45-µ filter. 0.3 ml of this solution was spiked in three different separating funnels with 0.1, 0.2 and 0.3 ml previously analyzed standard stock solution. Then 2.0-ml buffer, 2.0-ml dye and 10-ml chloroform was added and shaken for 2 min and allowed to stand for the separation of aqueous and organic layer. The lower organic layer of chloroform with ion-pair was collected in 10-ml volumetric flask and final volume was made up with chloroform. Estimation of drug content was done by proposed method.

Urimax capsules were weighed accurately. The capsule content was emptied and weight of empty capsule shells was taken. The difference of whole capsule and empty shells gave the weight of granules. The granules were powdered and weight equivalent to 6.25-mg TAM was transferred to 25-ml volumetric flask. Range

Linearity range of the proposed method was calculated by plotting response factor vs. concentration found to be 7.5-22.5µg/ml. Working range is found to be between 0.01 and 22.5 µg/ml and the test concentration of the method is 12.5 µg/ml.

Precision

Method was also validated in terms of repeatability, interday and intraday precision and RSD observed was 0.362, 0.489 and 0.997, respectively. ANOVA performed between the readings of interday and intraday precision showing no significant difference between them (Fcrit =3.438, Frows =3.43 and Fcolumn =5.31).

Recovery studies

Studies were performed with two different formulations veltam tablets (Intas) and urimax capsules (Cipla).

Results of recovery studies for Veltam tablet and Urimax are shown in the Table 6 and 7 respectively.

Limit of quantification and limit of detection

Limit of detection (LOD) and Limit of quantification (LOQ) was calculated by taking absorbance of six replicates of blank, calculating and substituting the SD and the value of slope from calibration curve using formula:

LOD = 3.3×(SD/Slope)
LOQ = 10×(SD/Slope)
LOD and LOQ of the method were found to be 0.003 and 0.01 µg/ml, respectively.

**Stochiometric of reaction**

Authors of the presented work try to establish stochiometry of reaction by mole ratio method and Job’s method of continuous variation.\(^{[25-27]}\)

2.10 \(^{-4}\)M solution of TAM and dye were prepared by dissolving 44.5-mg TAM in methanol and 67-mg BPB in distilled water, respectively, final volume was made up to 100 ml, this gave 10 \(^{-3}\)M solution. Ten milliliters of this solution was further diluted upto 50 ml with their respective solvents to obtain solution of 2.10 \(^{-4}\) molar strength.

**Mole ratio method**

2.10 \(^{-4}\)M TAM standard solution was transferred in seven separating funnel in a constant volume 2 ml, then 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of 2.10 \(^{-4}\)M dye solution was transferred from the 1st to the 7th separating funnel followed by 2-ml buffer and 10-ml chloroform. Shaken for 2 min and allowed to stand for 5 min for separation of two layers, the organic layer were collected in 10-ml volumetric flask marked 1-7 and final volume was made up to the mark with chloroform. The absorbance of formed chromophore was measured against chloroform and curve of absorbance was plotted against molar ratio of drug and total molar concentration of drug and dye [Figure 7]. Absorbance increases upto ratio 1 and becomes constant, proves that the drug binds with the dye in a ratio of 1:1.

**Job’s method of continuous variation**

The stochiometric ratio of TAM to BPB in the complex was determined by Job’s method of equimolar solutions. TAM standard solution 2.10 \(^{-2}\) M was pipetted into seven separating funnel (0, 0.5, 1, 1.5, 2, 2.5, 3, mL) and an aliquot of 2.10 \(^{-2}\) M BPB (3, 2.5, 2, 1.5, 1, 0.5, 0 mL) was added, respectively, keeping the mole ratio constant. Then 2-ml buffer and 10-ml chloroform was transferred and similarly shaken and allowed to stand for 2 and 5 min, respectively. The lower layer of chloroform was collected in 10-ml volumetric flask and final volume was made up to the mark with chloroform. The absorbance was taken against chloroform and a curve was plotted against absorbance and mole ratio of drug [Figure 8]. The absorbance increases upto 0.5 molar ratio with a positive slope shows that till there TAM was a limiting factor after that change in slope from positive to negative shows that dye was a limiting factor. Thus, the change in slope at 0.5 molar ratio conclude that the drug reacts with dye in 1:1 ratio.

The above two methods proves that the ratio of drug and dye in the reaction was 1:1 and dependency of reaction on buffer confirms that conversion into ionized form is also very necessary for the reaction. Thus, first the dye benzonoid form (blue color) of dye ionized into quinonoid form (purple color) in presence of buffer and reacts with protonated form of drug in 1:1 ratio and forms an ion-pair complex (yellow color). Figure 9 represents the proposed mechanism of reaction between drug and dye.

**Estimation of TAM in dosage form**

Powdered Veltam tablet equivalent to 6.25 mg of TAM was taken in 25-ml volumetric flask and

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Conc. found (mg)</th>
<th>Mean(mg) ± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veltam</td>
<td>3.89</td>
<td>3.89±0.045</td>
<td>1.1582</td>
</tr>
<tr>
<td>Urimax</td>
<td>4.05</td>
<td>3.977±0.064</td>
<td>1.61671</td>
</tr>
</tbody>
</table>

Figure 7: Mole ratio method of TAM-BPB ion-pair complexes.

Figure 8: Mole ratio method of TAM-BPB ion-pair complexes.
ultrasonication was done using approximately 20-ml methanol and diluted up to the mark with same. The content of drug in tablet was calculated by using regression equation.

For estimation in Urimax capsule, 20 capsules were weighed accurately, their contents were emptied in a petridish and ground in a mortar and pestle. The empty shells of 20 capsules were weighed and the difference in weight of whole capsules and empty shells gave the weight of granules. Powdered granules equivalent to 6.25 mg of TAM was taken in a volumetric flask and same procedure was followed as for Veltam tablets [Table 8].

CONCLUSIONS

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. In this case also the sensitivity of TAM was also increased to a great extent. The developed method was validated in terms of specificity, linearity, precision, accuracy and robustness. Table 9 presents optical and regression characteristics of the proposed method. Limit of detection and limit of quantification was found to be 0.003 µg/ml and 0.01 µg/ml, respectively, recovery studies shows that method is capable to recover analyte from both type of formulation i.e., tablet and capsule. RSD of interday and intraday precision is within acceptable limit of 2% proves that method is precise. Robustness studies were also performed by varying instrument and analyst. No significance difference was found between analysts and instruments at 5% significance level. Hence, it is evident that developed method can be used in pharmaceutical industries for routine quality control of Tamsulosin Hydrochloride in both capsules and tablets.

ACKNOWLEDGMENT

The authors are thankful to Aurobindo Pharmaceuticals, Hyderabad, for providing gift sample of Tamsulosin hydrochloride. We are also thankful to B.R. Nahata College of Pharmacy to provide facilities for the research.

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Source of Support: Nil, Conflict of Interest: None declared.