Original Article

Spectrophotometric estimation of tamsulosin hydrochloride by acid-dye method

Abstract

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 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).

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

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INTRODUCTION

Tamsulosin hydrochloride 5-[(2R)-2-[2-(2-ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzenesulfonamide hydrochloride [Figure 1] is a uroselective α_{1A} ($\alpha_{1A:}$ α_{1B} affinity 7-38-fold) antagonist which is used in benign prostatic hyperplasia (BPH). The α_{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]

Various analytical methods reported are HPLC-UV method for estimation of TAM and its impurity (J.G. Chandorkar *et al*.),[3] LC/ESI-MS–MS method (R. Nageswara Rao *et al*.)[4] for assay and related substance estimation, LC-MS for determination of tamsulosin in human aqueous humor and serum (Pekka Keski-Rahkonen *et al*.).[5] In plasma estimation by LC–ESI-MS reported by Li Ding *et al*.,[6] estimation of drug in dog plasma by LC-MS,[7] chiral separation by its *S*-isomer by HPLC-UV[8] and HPLC with fluorescence estimation in human plasma[9] is also reported. Other methods include voltametry[10] and chiral separation by capillary electrophoresis[11] is also available in the literature. HPTLC[12] and radioreceptor analysis[13] of TAM alone and in combination with 5 α_1-reductase inhibitor like dutasteride[14] and finasteride[15] such as UV spectroscopy, ratio derivative spectroscopy, LC–MS–MS[16], HPLC-UV[17] and LC-TMS[18] methods are also developed and reported so far. Methods in combination with tolterodine tartrate by UV[19] and HPLC-UV[20] methods are available in the current scientific communications. But to the best of our knowledge there is no single method

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 λ_{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 µg/ml. 2.5 ml of this stock was diluted 50 ml to obtain a working standard of 250 µg/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

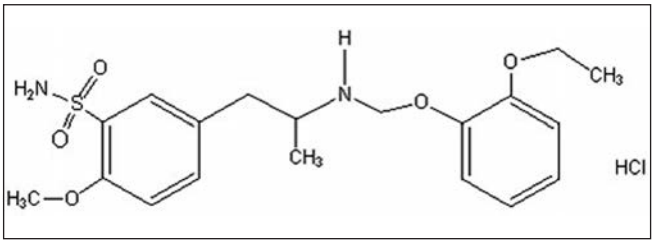

Figure 1: Chemical structure of tamsulosin hydrochloride

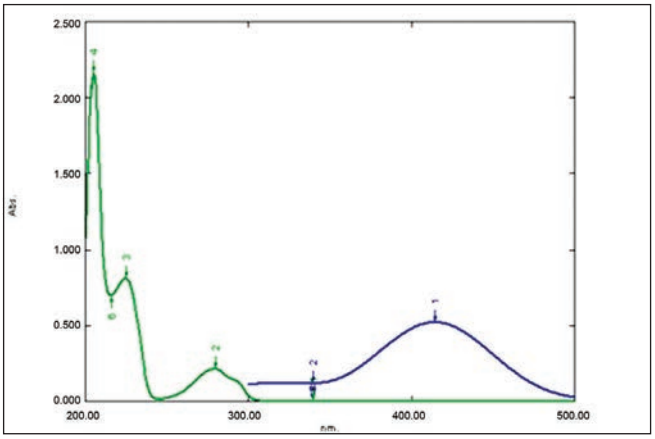

Figure 2: Overlay spectra of pure TAM (a) 200 µg/ml in methanol and (b) Ion-pair complex (10 µg/ml with BPB in chloroform)

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 upto 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 6] 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.

Table 1: Summary of optimization studies performed

Experiment no.	pH of buffer	Volume of buffer (ml)	Inference
1.	2.2	1	No chromophore formation
		2	No chromophore formation
		3	No chromophore formation
		4	Absorbance was less
2.	2.1	1	No chromophore formation
		2	No chromophore formation
		3	Absorbance was less
		4	Absorbance was less
3.	2.0	1	Absorbance was less
		2	Maximum absorbance and stability of chromophore
		3	Good absorbance but less stability
		4	Good absorbance but less stability

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.

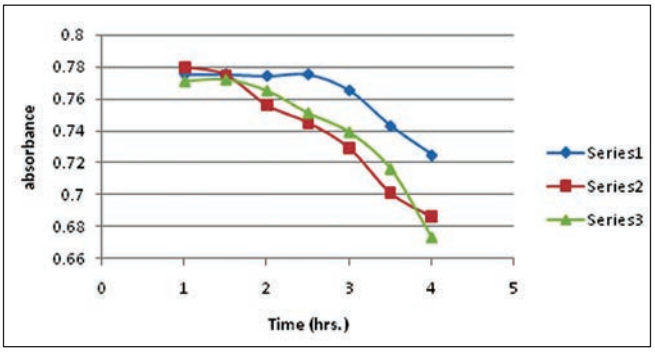

Figure 3: Stability of chromophore (experiment 3-volume 2, 3, 4 ml)

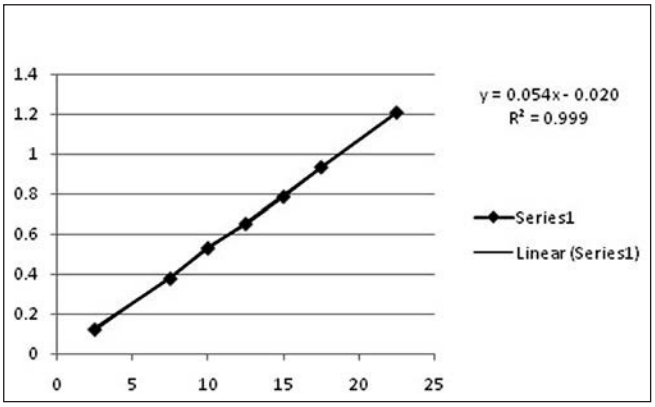

Figure 4: Calibration curve of TAM

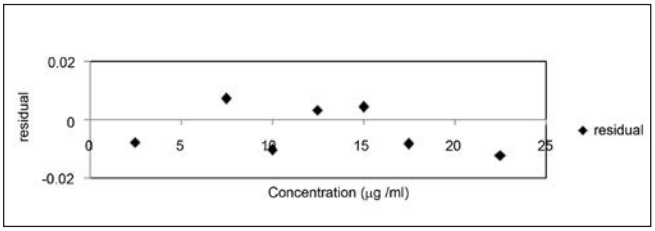

Figure 5: Plot of residual vs, concentration

Linear function analysis: Linear function analysis or lack of fitness test is applied by calculation of SS_r, $SS\epsilon$, 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]

Table 2: Ascending series of data of calibration curve

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

Table 3: Data of Dixon test for outliers

Conc. (µg/ml)	r smallest	r largest
2.5	0.3571	0.21429
7.5	0.2143	0.28571
10	0.3333	0.25
12.5	0.4444	0.08889
15	0.3125	0.1875
17.5	0.25	0.28571
22.5	0.2353	0.05882
Limit at 5% significance level	0.560	

$$SSr = \sum_{i=1}^{i} \sum_{j=1}^{ii} (yij - \hat{y}i)^2 = 0.005215$$

$$SSlof = SSr - SS\epsilon = \sum_{i=1}^{i} (\bar{y}i - \hat{y}i)^2 = 0.004529$$

$$SS\epsilon = \sum_{i=1}^{i} \sum_{j=1}^{ji} (yij - \bar{y})^2 = 0.000686$$

6.1.5.2.4.2 Calculation of degrees of freedom:

$DF_r = (IJ - 2) = 34$

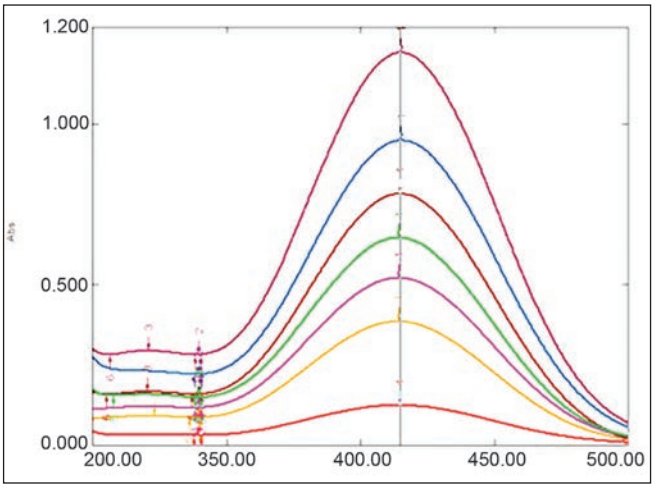

Figure 6: Overlay spectra of TAM (2.5, 7.5, 10, 12.5, 15, 17.5 and 22.5 µg/ml) by the proposed method

Table 4: Data of residual error sum squares

X	$(yi-\hat{y}i)^2$					
2.5	3.025E-05	0.000001	2.5E-05	9E-06	0.00021	0.00021
7.5	0.0002102	0.000361	2.5E-05	1.6E-05	2.25E-06	0.00024
10	6.4E-05	0.000289	0.000289	4.9E-05	6.4E-05	3.6E-05
12.5	0.0001323	0.0001	0.000001	0.0001	0.000992	5.63E-05
15	0.000289	1E-04	8.1E-05	4.9E-05	0.000144	1.6E-05
17.5	2.5E-07	4E-06	9E-06	0.000324	3.03E-05	6.25E-06
22.5	1.225E-05	1E-06	0.000256	4.9E-05	0.00024	9.03E-05

Table 5: Data of pure error sum squares

X	$(yi-\bar{y})^2$					
2.5	3.80278E-05	6.14E-05	4.69E-06	6.94E-07	8.03E-06	1E-05
7.5	1.46944E-05	0.000165	5.14E-05	0.00023	1.36E-06	4.67E05
10	1.77778E-06	3.21E-05	0.000136	4.44E-07	1.88E-05	0.000152
12.5	1E-04	8.1E-05	1.6E-05	3.6E-05	0.000961	0.000196
15	0.000261361	3.8E-05	6.94E-07	0.000251	1.74E-05	9.67E-05
17.5	0.000205444	5.38E-05	1.11E-07	0.000187	7.11E-06	3.21E-05
22.5	0.000427111	0.00016	6.94E-05	2.78E-06	0.000178	0.000178

Table 6: Recovery studies of Veltam tablets

Conc. (µg/ml)	Conc. found before Spiking (µg/ml) C_1*	Conc. of Std. added C_2*	Conc. found after Spiking (µg/ml) C_3*	% Recovery (C_3-C_1) *100/C2	Mean + SD	RSD
7.5	7.62963	2.62963	10.2037	98.00	100.16 ± 0.0199	0.0198
		5.2963	12.98	101.05		
		7.805	15.6481	101.56		

*Mean of 3 determinations

Table 7: Recovery studies of Urimax capsules

Conc. (µg/ml)	Conc. found before Spiking (µg/ml) C_1*	Conc. of Std. added C_2*	Conc. found after Spiking (µg/ml) C_3*	% Recovery (C_3-C_1) *100/C2	Mean + SD	RSD
7.5	7.7963	2.62963	10.41	99.296	99.09 ± 0.0036	0.0036
		5.2963	13.056	99.301		
		7.805	15.498	98.677		

*Mean of 3 determinations

$DF_{\epsilon} = (IJ - I) = 30$

$DF_{lof} = (I - 2) = 4$

Calculation of associated variance

$$\sigma_r^2 = \frac{SSr}{DFr} = 0.000153$$

$$\sigma_e^2 = \frac{SS\varepsilon}{DF\varepsilon} = 0.000151$$

$$\sigma_{lof}^2 = \frac{SSIof}{DFlof} = 0.000172$$

Acceptability of linearity data

F ratio = $\sigma_\epsilon^2 / \sigma_{lof}^2 = 0.879$

Result: F tabulated at 95% confidence level is 2.69 and F calculated is 0.879, thus F calculated < F tabulated therefore the method is linear.

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 (F_{crit}=3.438, F_{rows}=3.43 and F_{column}=5.31).

Recovery studies

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

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 and same procedure was followed for Veltam tablets.

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 stochiometery 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 pippetted 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

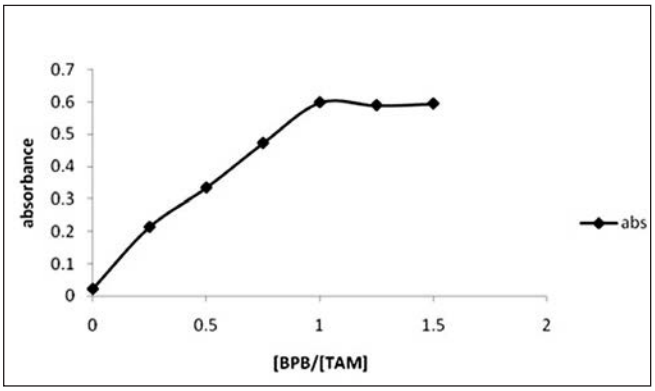

Figure 7: Mole ratio method of TAM-BPB ion-pair complexes.

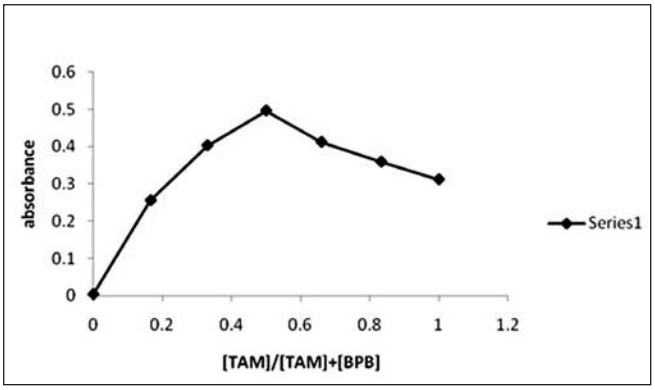

Figure 8: Mole ratio method of TAM-BPB ion-pair complexes.

Table 8: Estimation of TAM

Formulation	Conc. found (mg)	Mean(mg) ± SD	RSD
Veltam	3.89	3.893±0.045	1.1582
	3.85		
	3.94		
Urimax	4.05	3.977±0.064	1.61671
	3.95		
	3.93		

ultrasonication was done using approximately 20-ml methanol and diluted upto 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 grounded 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 spectrophotmetery 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.

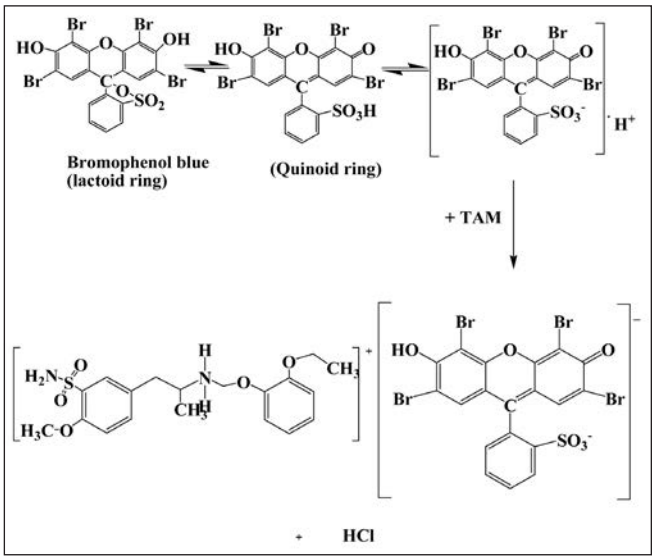

Figure 9: Proposed mechanism of reaction of TAM and BPB by proposed method

Table 9: Optical and regression characteristics of the proposed method

Parameters	Inference
λmax (nm)	414 nm
Beer's law limit (µg/ml)	2.5 – 22.5 µg/ml
Sandell's sensitivity (µg.cm2 per 0.001 absorbance unit)	0.0192
Molar absorptivity	2.32×104
Linear Regression equation	y = 0.054x – 0.020
Correlation coefficient (r2)	0.999

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