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Spectrophotometric Method for Estimation of Tamsulosin Hydrochloride in Pharmaceutical Dosage Form Using Bromate-Bromide and Methyl Orange Reagent

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ABSTRACT

A simple, rapid, accurate and precise assay procedure based on Spectrophotometric method has been developed for the estimation of Tamsulosin hydrochloride in Pharmaceutical formulation. The method was based on the bromination of Tamsulosin hydrochloride with a known excess amount of Bromate-bromide mixture in acidic medium followed by the determination of surplus bromine by reacting with dye methyl orange and measuring the absorbance at 513 nm. Validation was carried out in compliance with International Conference on Harmonization guidelines. Linear regression analysis of method showed good linearity with the correlation co-efficient (r) of 0.9978 with respect to absorbance in the concentration range of 2-12 µg/mL and the mean recovery for Tamsulosin hydrochloride was 99.65% ± 0.47 . The proposed method can be successfully applied for the analysis of tablet formulations.

KEYWORDS

Tamsulosin hydrochloride, Spectrophotometry, Bromination.

INTRODUCTION

Tamsulosin hydrochloride (TAM), (-) - (*R*) – 5 - [2 - [[2 - (*O* – Ethoxy phenoxy) ethyl] amino] propyl] – 2 – methoxy benzene sulfonamide hydrochloride. The chemical structure is shown in Fig. 1 is a highly selective α-antagonist used for the treatment of patient with symptomatic benign prostatic hyperplasia. It is selective for α_{1A} and α_{1D} receptors, which are predominant in the prostate, prostatic capsule, prostatic urethra and bladder. The relaxation of prostate and bladder smooth muscles is associated with improved maximal urine flow and reduction of lower urinary tract symptoms[1-3].

Literature survey revealed that several bioanalytical methods have been reported for analysis of TAM, These include HPLC and determination in human plasma[4], plasma dialysate and urine[5], human aqueous humor and serum using liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS-MS)[6,7], TAM in dog plasma was also determined by LC-MS-MS[8]. Other methods included use of electrophoresis and HPLC for chiral separation[9,10]. RP-LC/ESI-MS-MS method was reported for separation, identification and determination of TAM in bulk drugs and formulation[11]. Recently, stability indicating HPTLC method was reported for determination of TAM in formulation[12].

To the best of author's knowledge, a visible spectroscopic method based on colour development not yet been reported for estimation of TAM. So, it was thought of interest to develop such an approach for the quantitative analysis of TAM in pharmaceutical formulation.

The objective of this work was develop and validate inexpensive, simple and rapid spectrophotometric method for determination of TAM in pharmaceutical formulation which

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would be accurate and precise. The method involves treating a fixed amount of bromate-bromide solution in acid medium with TAM solution and determining the unreacted bromine by treating with a fixed amount of methyl orange dye solution and measuring absorbance at 513 nm. The proposed methods have the advantages of sensitive and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

MATERIAL AND METHOD

Instruments and Apparatus

A Shimadzu (Kyoto, Japan) model UV-1700 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Sartorius CP224S analytical balance (Gottingen, Germany) and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Reagents and Chemicals

All the reagents used during the study were of analytical grade.

Methyl Orange Dye Solution: (50 µg/mL)

Prepared by dissolving accurately weighed 5 mg Methyl Orange dye (S.D. Fine Chemicals Ltd, Mumbai) in distilled water and diluting to the mark in 100 mL volumetric flask.

Bromate-bromide Solution: (30 µg/mL with respect to $KBrO_3$)

Prepared by dissolving accurately weighed 10 mg of $KBrO_3$ (Finar Chemicals Ltd, Mumbai) and 133 mg of KBr (Finar Chemicals Ltd, Mumbai) in distilled water and diluting to the mark in 100 mL volumetric flask having concentration 100 µg/mL with respect to $KBrO_3$.The solution was appropriately diluted to obtain 30 µg/mL solution with respect to potassium bromate.

Hydrochloric Acid Solution (1M)

Prepared by diluting 8.9 mL of concentrated HCl (Merck, India) in distilled water in 100 mL volumetric flask.

Standard Stock Solution of TAM (100 µg/mL)

Tamsulosin hydrochloride pure powder was kindly gifted by Cadila Healthcare Ltd., Ahmadabad. An accurately weighed quantity of about 10 mg TAM was transferred to a 100 mL volumetric flask dissolve in and diluted up to the mark with methanol (Ranbaxy Fine Chemicals Ltd, New Delhi, India).

Sample Solution of TAM

To determine the content of TAM in Tablet (Veltam 0.4 mg, modified release tablets, Intas Pharmaceuticals, Dehradun, India) were purchased from the local pharmacy.30 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of TAM was transferred into a 50 ml volumetric flask containing 20 ml methanol, sonicated for 30 min. Finally volume was made up to the mark with methanol and further shaken for 15 min for complete extraction of TAM from its matrix. The resulting solution was centrifuged for 20min at 4,000 rpm and the supernatant was diluted with methanol to obtain 50 µg/ml of TAM and filtered through Whatman No.42 paper (Whatman International Ltd., England). Suitable aliquot of filtrate was analyzed by the method.

General Procedures

Different aliquots of standard TAM solution (0.2- 1.2 mL,100 µg/mL) were accurately transferred into a series of 10 mL volumetric flasks. To each flask was then added 1 mL of 1M HCL followed by 1mL of 30 µg/mL Bromate-bromide solution (w. r. t. $KBrO_3$). The flasks were stoppered, contents mixed well and let stand for 20 min. with occasional shaking. Then 2 mL of 50 µg/mL methyl orange dye solution was added to each flask, the volume adjusted to the mark with methanol, mixed well

and absorbance of each solution was measured at 513 nm against reagent blank.

Method Validation

The Spectrophotometric method was validated as per ICH guidelines[13] .

Linearity

The linear response of TAM was determined by analyzing different concentration levels of the calibration curve in the range of 2-12 µg/mL for TAM. The standard solutions for linearity were prepared 5 times.Result should be expressed in terms of Correlation co-efficient.The calibration curves were constructed by plotting absorbance areas versus concentrations.

Accuracy (% Recovery)

Accuracy of the method was determined by standard addition method in which the known amount of standard TAM solutions were added to preanalyzed tablet solution. These amounts corresponded to 50, 100 and 150 % of the amounts claimed on the label. The amounts of TAM were estimated by applying these values to the regression equation of the calibration curve.

Method Precision (Repeatability)

It was performed by measuring absorbance of six different working standard solutions of TAM for the same concentration (4 µg/mL). Repeatability is reported in terms of relative standard deviation (RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating 3 times on the same day and on 3 different days for 4 different concentrations of calibration range of TAM (2, 6, 10 and12 µg/mL) . The results are reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ of TAM were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response

S = slope of calibration curve

Analysis of TAM in Tablets

To determine the content of TAM in conventional tablets, 30 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of TAM was transferred into a 50 ml volumetric flask containing 20 ml methanol, sonicated for 30 min. Finally volume was made up to the mark with methanol and further shaken for 15 min for complete extraction of TAM from its matrix. The resulting solution was centrifuged for 20min at 4,000 rpm and the supernatant was diluted with methanol to obtain 50 µg/ml of TAM and filtered through Whatman No.42 paper. Suitable aliquot of filtrate was analyzed by the method.

RESULTS AND DISCUSSION

Many dyes are prone to oxidation to form colorless products in acid medium, thus offering a suitable analytical approach for the inherent Spectrophotometric determination of different Pharmaceutical substances using oxidizing agent including in *situ* generated bromine[14-19]. An acidified mixture of bromate behaves as an equivalent solution of bromine and has been extensively used for the determination of inorganic and organic substances. This mixture has been widely used in the assay of a number of substances of pharmaceutical importance by indirect spectrophotometry based on either redox or substitution reactions.

Method Development

In the present investigation, the reaction between bromine and methyl orange dye was used for the indirect Spectrophotometric

determination of TAM. In the proposed method, different amounts of TAM were reacted with a fixed and excess amount of the bromate-bromide mixture in acidic medium, and after a predetermined time ,the unreacted bromine was determined by treating with a fixed amount of Methyl orange dye solution and measuring the absorbance at 513 nm, as shown in Figure-1. The absorbance was found to increase linearly with the concentration of TAM.

Preliminary study showed that 2 mL of 50 µg/mL (Figure-2) methyl orange acid form in a total volume of 10 mL produced a convenient maximum absorbance at 513 nm. This colour was completely and irreversibly bleached by 1 mL of 30 µg/mL of bromate (with respect to $KBrO_3$) in the presence of excess bromide in acid medium.

Hence, different amount of TAM were reacted with of 1 mL of 30 µg/mL bromate (with respect to $KBrO_3$) in acid medium and in the presence of excess bromide, after the bromination was judged complete, the surplus bromine (in situ generated) was determined by treating with 2 mL of 50 µg/mL of Methyl orange, measuring the absorbance at 513 nm. This step enabled to determine the concentration range of TAM which could be quantitated. The possible reaction Scheme is given in Figure-3.

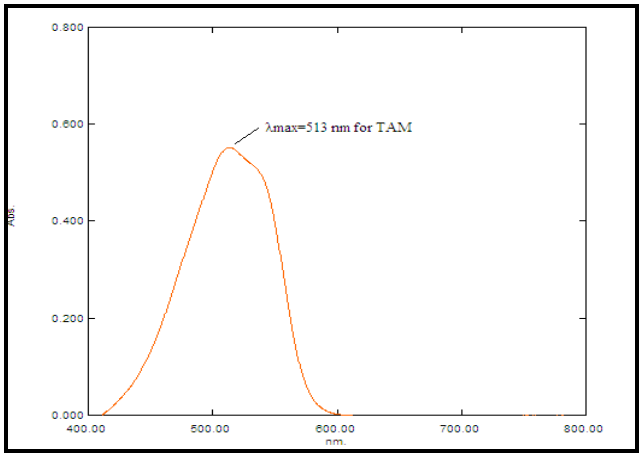
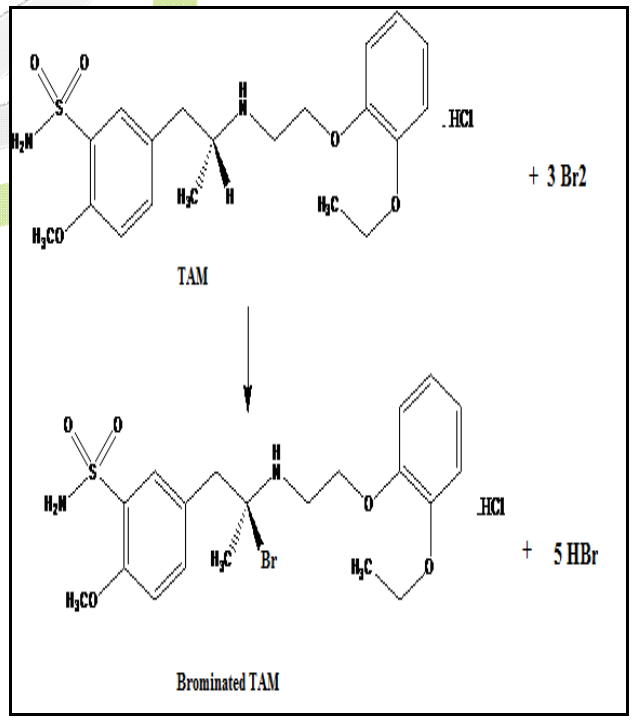


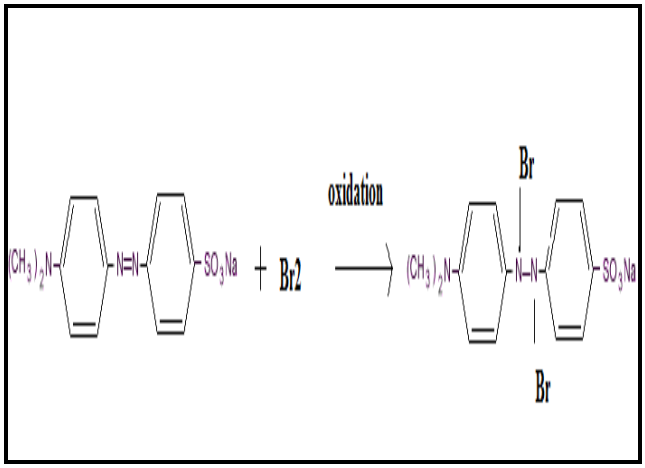
Figure 1: Representative absorption spectra of TAM -MO showing λmax at 513 nm

TAM, when added in increasing amounts to a fixed amount of bromate-bromide mixture in acid medium, consumes bromine proportionately, and there will be concomitant fall in bromine concentration. When fixed amount of Methyl Orange was added to decreasing concentration of bromine, a proportional increase in dye concentration occurred as indicated by proportional increase in absorbance at 513 nm as a function of TAM concentration. Hydrochloric acid medium was ideally suited for both bromination and bleaching steps. One mL of 1M HCL was found to be adequate for the bromination reaction (Figure-4), which was complete in 20 min (Figure-5), and the same concentration was maintained for the determination of the residual bromine by its bleaching action on Methyl orange. Contact time of 20 min was not critical and any delay up to 40 min in the determination of the residual bromine had no effect on the absorbance. The measured color is stable for several days.

$$BrO_3^- + 5\,Br^- + 6\,H^+ \rightarrow 3\,Br_2 + 3H_2O$$



Bromination of TAM



Methyl Orange (Orange Color) Colorless Product (In acidic medium)

Figure 2: Possible reactions scheme for bromination of TAM

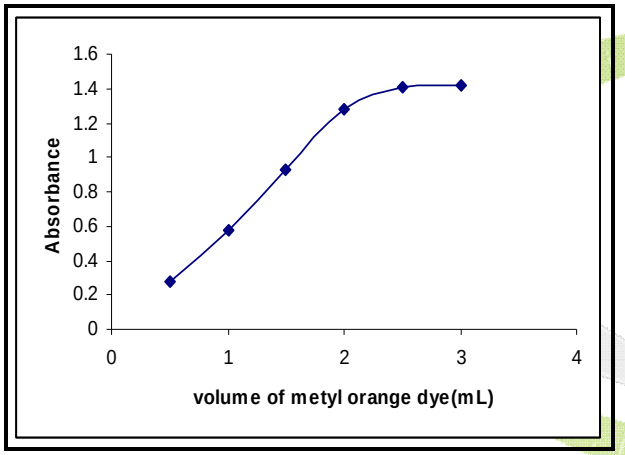


Figure 3: Optimization of Volume of Methyl Orange (50 µg/mL)

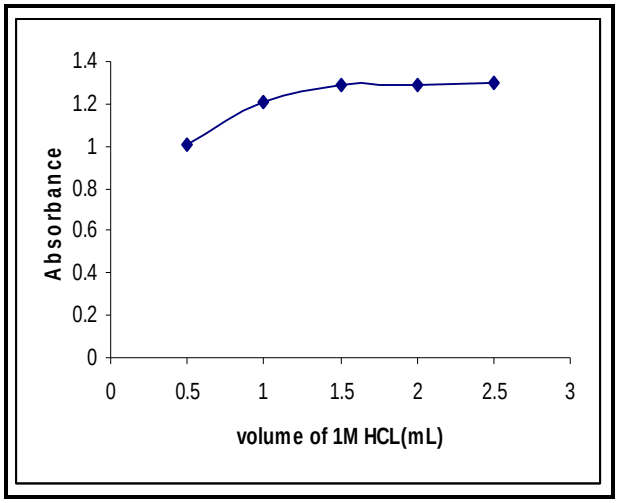


Figure 4: Optimization of Volume of 1M HCL

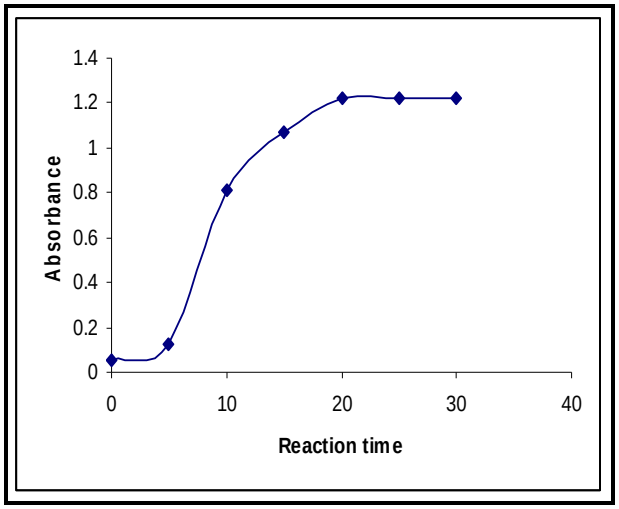


Figure 5: Optimization of Reaction time

Validation of the Method

The described method has been validated for linearity, accuracy and intermediate precision. The standard solutions for linearity were prepared 5 times at different concentration levels. The calibration curve was found to be linear in the range of 2-12 µg/mL with Correlation Coefficient (r) of 0.9978. The limit of detection (LOD) and limit of quantification (LOQ) were determined by calculating method based on the standard deviation of response and the slope as suggested in ICH guidelines, which were found to be 0.57 and 1.74, respectively. Sensitivity parameters such as molar absorptivity (L/mol.cm) and Sandell's sensitivity (µg/cm^2 /0.001 Absorbance units) were found to be 37,781 and 0.0108, respectively. Repeatability of measurement of absorbance was expressed as RSD and was 0.247 for six replicate determinations. The low values of RSD indicate that the proposed method is repeatable. The intraday and interday variations for the determination of TAM were evaluated at 4 different concentration levels (2, 6, 10 and 12 µg/mL). The RSD value obtained for intra-day and inter-day variation were 0.18 - 1.12 % and 0.14 -1.48 % respectively revealed that proposed method was precise. Regression analysis data and summary of all validation parameters are given in Table 1. Accuracy was determined on previously analyzed formulation

after spiking with 50, 100 and 150% of the additional of the standard TAM (Table 2). Mean recovery obtained was 99.65 ± 0.47 %.

Analysis of Formulated Tablets

The tablet formulation was analyzed by developed method and assay result was found to be 99.62 % of the labeled claim with standard deviation 0.72 (Table 3). There was no interference from the excipients which might have been present in the tablet; hence the proposed method is applicable for the routine estimation of TAM in pharmaceutical dosage forms.

Table 1: Summary of validation parameters of proposed spectrophotometric method

Parameters	TAM
Linearity (µg/mL)	2-12
Molar absorptivity (L/ mol cm)	37,781
Sandell's	0.0108
Regression equation y=mx+c*	y = 0.1123x
Slope (m)	0.112
Intercept (c)	0.0985
Correlation coefficient (r^2)	0.9978
LOD (µg/mL)	0.57
LOQ (µg/mL)	1.74
Accuracy (% recovery) (n=3)	99.65 ± 0.47
Repeatability (% RSD) (n=6)	0.247
Precision (% RSD)	
Interday (n = 4)	0.14 -1.48
Intraday (n = 4)	0.18 -1.12

* y = mx + c, x = concentration

CONCLUSION

The proposed spectrophotometric method has linear response in the stated range and is accurate, precise and sensitive. The striking advantages of spectrophotometric the high sensitivity using inexpensive instrument as compared to those used in many reported analytical techniques for assay. The method is rapid and do not involve heating or extraction step and any stringent experimental condition which influence the sensitivity and reliability of method. In terms of sensitivity, simplicity and conveniently the method can be used for routine quality control of tablet formulations.

Table 2: Multilevel recovery study of tam

Level of Recovery (%)	Amt of sample taken (µg/mL)	Amt of std spiked	% Recovery	Mean % Recovery ± SD (n=3)
50	4.0	2.0	99.95	99.31 ± 0.62
			98.70	
			99.30	
100	4.0	4.0	99.45	99.71 ± 0.26
			99.72	
			99.97	
150	4.0	6.0	99.33	99.94 ± 0.53
			100.16	
			100.33	

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Table 3: Analysis of marketed tablet formulation of tam by proposed method

No.	Labeled claim TAM (mg/tab)	Amount found TAM(mg/tab)	%Assay
1	0.4	0.398	99.50
2	0.4	0.399	99.75
3	0.4	0.394	98.50
4	0.4	0.402	100.5
5	0.4	0.397	99.25
6	0.4	0.401	100.25
Mean			99.62
SD			0.72

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