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Ion-Pair Complex Method for the Spectrophotometric Determination of Tamsulosin In the Presence of Dutasteride by using Bromo Thymol Blue

I. Lakshmi Prasanna*[1], G.T. Naidu[1], Nuzhath Fathima[2], I.E.Chakravarthy[2] and G.Abdul Huq[3]

1. Department of Physics, Rayalaseema University, Kurnool.518007, AP.
2. Department of Chemistry, Rayalaseema University, Kurnool.518007, AP
3. Department of Chemistry, School of Sciences, Maulana Azad National Urdu University, Hyderabad, 500032, TS.

ABSTRACT

A simple, rapid, sensitive, accurate, precise spectrophotometric method has been developed for the estimation of Tamsulosin in the presence of Dutasteride in pharmaceutical formulations. During the course of study, it is observed that acidic solution of the drug formed colored ion-association complexes with Bromo Thymol Blue (BTB) which is soluble in methanol. This property of the drug was followed for the development of spectrophotometric method for analysis of drug. The complex of Tamsulosin in the presence of Dutasteride with Bromo Thymol Blue showed λ_{max} at 430 nm. The linearity range for Tamsulosin in the presence of Dutasteride was 10 µg/ml to 250 µg/ml. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. The molar absorptivity and the sandell sensitivity of the method are found to be 6.6123×10^4 lit/mole/cm and 0.0067 µg/ml/cm^2 respectively.

Keywords: Spectrophotometry, Tamsulosin, Dutasteride, Ion-pair complex, Bromo Thymol Blue, Pharmaceutical formulations.

*Corresponding Author Email: prasannainguva@gmail.com
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INTRODUCTION

Tamsulosin (TAM), chemically 5-[(2R)-2-{[2-(2- ethoxyphenoxy) ethyl] amino} propyl]-2-methoxybenzene-1- sulfonamide[1-4], is a white crystalline powder and is freely soluble in methanol, acetonitrile, ethanol and partially insoluble in water. Categorized as antineoplastic agents, adrenergic alpha-Antagonists. Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. Route of elimination of tamsulosin hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver and less than 10% of the dose is excreted in urine unchanged. %). Half-life of drug is 4 weeks. The structure of Tamsulosin is given below in figure 1.

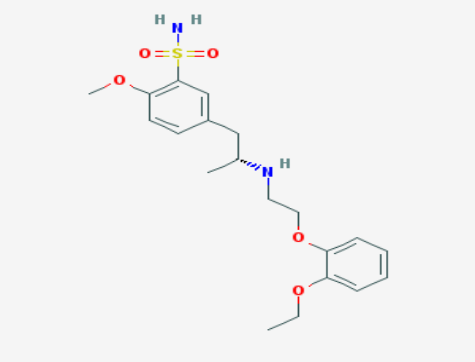

Figure 1: Structure of Tamsulosin

Dutasteride(DUTA)Chemically(1S,2R,7R,10S,11S,14S,15S)-N-[2,5bis(trifluoromethyl) phenyl]-2,15-Dimethyl-5-oxo-6zatetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadec-3-ene-14-carboxamide [5-13], is a white powder and is freely soluble in acetonitrile, ethanol, methanol and partially insoluble in water.Route of elimination of Dutasteride is extensively metabolized in humans and excreted mainly in feces, Protein binding of albumin (99%) and α-1 acid glycoprotein (96.6%). Half-life of drug is 5 weeks. The structure of Dutasteride is as figure 2 given below. Combination therapy as a fixed-dose Dutasteride & Tamsulosin for lower urinary tract symptoms secondary to benign prostatic enlargement, which is composed of two active ingredients, Tamsulosin and Dutasteride. Tamsulosin is a α-adrenoceptor blocker that is relatively selective for the α (1A)-adrenoceptor subtype within the prostatic smooth muscles. The inhibition of α (1A)-adrenoceptor results in smooth muscle relaxation. Dutasteride is an inhibitor of 5α-reductase, an enzyme that is responsible for the conversion of testosterone to its active form dihydrotestosterone. This occurs in the prostate, liver and skin. 5α-Reductase results in the shrinkage of the prostatic epithelium and reduction in the size of the prostate.

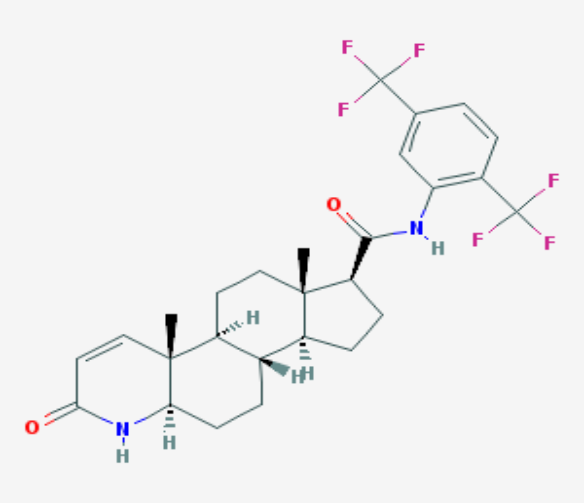

Figure: 2 Structure of Dutasteride

No clinical studies have been performed on the fixed-dose Dutasteride/Tamsulosin combination, although several clinical trials have been conducted on the combination therapy of 5α-reductase and α-adrenoceptor blockers. The combination therapy was associated with significant improvements in the symptom compared to Tamsulosin or Dutasteride as monotherapy. It is therefore logical to combine the two medications into one tablet. Literature indicates RP-HPLC method was determination of TAM and DUTA in pharmaceutical formulations is reported, but stability indicating method by UV spectroscopy method was not yet reported for the simultaneous determination of TAM and DUTA.

MATERIALS AND METHOD

(A) Instruments used

Spectrophotometer:

A Single beam UV-Spectrophotometer Model SP-UV200 with 1 cm matched quartz cuvettes is employed throughout the study for all absorbance measurements.

pH Meter:

A digital ELICO-pH Meter Model LI-120 is used for pH measurements.

(B) Preparation of Reagents and Solutions

Tamsulosin solution:

50 mg of pure Tamsulosin is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 50 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

Dutasteride solution:

50 mg of pure Dutasterideis dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 50 µg /ml of the drug is prepared by suitably diluting the stock solution as and when required.

Bromo Thymol Blue solution (0.5% w/v):

Bromo Thymol Blue is prepared by dissolving 500 mg of Bromo Thymol Blue in 100 ml of distilled water.

Buffer solution pH 2.5 (Potassium acid Phthalate - HCl):

It is obtained by diluting a mixture of 50 ml of 0.2 M potassium acid phthalate and 8.4 ml of 0.2 M HC1 to 200 ml with distilled water and the pH is adjusted to 2.5 using the pH meter.

All other chemical substances and reagents employed in the present investigations are of AR Grade only.

RESULTS AND DISCUSSION:

Tamsulosin in the presence of Dutasteride when treated with Bromo Thymol Blue (BTB) forms a colored Ion pair complex. This Ion-Pair complex formation reaction is spectrophotometrically monitored to develop a method for the determination of purity of the drug. In this process a detailed investigation is done to know the optimization of various parameters such as wavelength of maximum absorbance (λ_{max}), the effect of concentration of Buffer solution (pH 2.5) and Bromo Thymol Blue on the absorbance of Ion Pair complex are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of Ion Pair Complex: -

The absorption spectrum of the Ion – Pair complex formed between Tamsulosin in the presence of Dutasteride and Bromo Thymol Blue is obtained in order to fix the wave length of maximum absorbance and its experimental procedure is as follows.

2 ml of Tamsulosin solution (100 µg/ml), 1 ml of Dutasteride solution (100 µg/ml), 2 ml of BTB solution (0.5% w/v), 3 ml of Buffer solution of pH 2.5 and 2 ml of methanol are taken in a 10 ml standard flask the resulting solution is made upto the mark with distilled water. Then the absorbance values of the Ion- pair complex formed are measured in the wavelength range 330 nm to 500 nm against the reagent blank. The results obtained are used to draw a graph between the wavelength and the absorbance values. The graph obtained is the absorption spectrum from which the wavelength of maximum absorbance is noted. This is shown (absorption spectrum) in following figure 3.

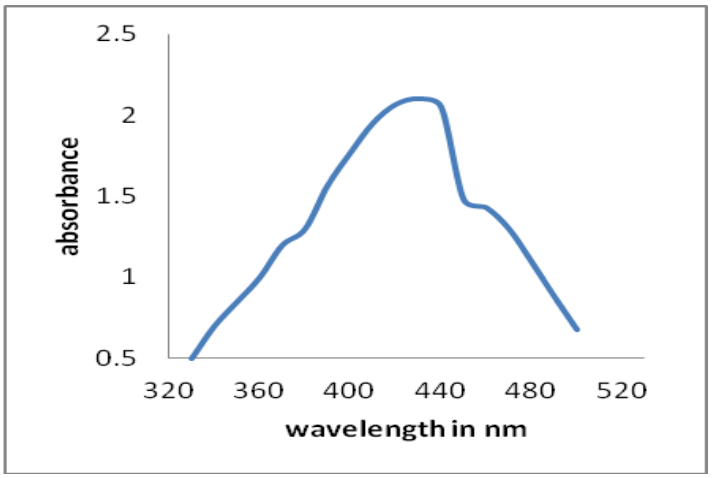

Figure 3: Absorption spectrum of Ion-Pair complex of Tamsulosin with BTB

It is seen from the below graph of the absorption spectrum, the maximum absorbance is obtained at 430 nm. Hence for all further studies a wavelength of 430 nm is fixed.

Effect of Volume of Buffer solution of pH 2.5:- The effect of Buffer solution of pH 2.5 on the absorbance of ion pair complex is studied by taking varying volumes (x ml) Buffer solution of pH 2.5 in a series of 10 ml standard flask.To each flask 10 ml now, 2 ml of Tamsulosin solution, 1ml of Dutasteride solution, 2.5 ml of BTB solution (0.5% w/v) and 1.5 ml of methanol are added followed by the addition of distilled water to make up each 10 ml flask to mark. The absorbance of each solution is recorded at 430 nm against the suitable blank. The results obtained are shown in Table 1.

2 ml Tamsulosin solution (100 µg/ml) + 1 ml of Dutasteride solution (100 µg/ml) + 2.5 ml of BTB solution (0.5% w/v) + x ml of Buffer solution (pH 2.5) + 1.5 ml of methanol + (3-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 430nm.**

Table 1: Effect of Volume of Buffer Solution of pH 2.5

S. No	Vol. of Tamsul-osin (100 µg/ml) in ml	Vol. of Dutasteride (100 µg/ml) in ml	Vol.of BTB (0.5% w/v) in ml	Vol. of Buffer Solution (of pH 2.5) x ml	Vol.of Methanol in ml	Vol. of distilled water in ml(3-x)	Total Vol. in each flask in ml	Absorbance
1	2.0	1.0	2.5	0.5	1.5	2.5	10	0.716
2	2.0	1.0	2.5	1.0	1.5	2.0	10	0.844
3	2.0	1.0	2.5	1.5	1.5	1.5	10	0.762
4	2.0	1.0	2.5	2.0	1.5	1.0	10	0.568
5	2.0	1.0	2.5	2.5	1.5	0.5	10	0.657

From the above Table 1, it is observed that 1.0 ml of Buffer solution of pH 2.5 is necessary to achieve maximum absorbance. Hence for all further studies a volume of 1.0 ml of Buffer solution of pH 2.5 is fixed.

Effect of Bromo Thymol Blue (BTB) concentration:

The effect of Bromo Thymol Blue on the absorbance of Ion – Pair complex is studied by taking varying volumes (x ml) of BTB in a series of 10 ml standard flasks. After taking x ml (0.5 ml to 2.5 ml) of BTB in each flask, 1.0 ml of Buffer solution of pH 2.5, 2 ml of drug solution of Tamsulosin solution , 1.0 ml of Dutasteride solution 1.5 ml of methanol are added and the resulting solution is made upto 10 ml using distilled water. The absorbance of each solution is recorded at 430 nm against a suitable blank. The results obtained are mentioned in below Table.2:

2 ml Tamsulosin solution (100 µg/ml) +1 ml of Dutasteride solution(100 µg/ml) + x ml (0.5 ml to 2.5 ml) of BTB solution (0.5% w/v) + 1.0 ml Buffer solution (pH 2.5) + 1.5 ml of methanol + (4-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 430 nm.**

Table 2: Effect of BTB on Ion- Pair complex

S. No	Vol.of Tamsulosin (100 µg/ml) in ml	Vol.of Dutaste-ride (100 µg/ml) in ml	Vol.of BTB solution (0.5% w/v) x ml	Vol.of Buffer Solution (pH 2.5) in ml	Vol.of Metha n-ol in ml	Vol.of distilled water in ml (4.5-x)	Total Vol. in each flask in ml	Absorb ance
1	2.0	1.0	0.5	1.0	2.0	4.0	10	0.303
2	2.0	1.0	1.0	1.0	2.0	3.5	10	0.529
3	2.0	1.0	1.5	1.0	2.0	3.0	10	0.826
4	2.0	1.0	2.0	1.0	2.0	2.5	10	1.076
5	2.0	1.0	2.5	1.0	2.0	2.0	10	1.303
6	2.0	1.0	3.0	1.0	1.5	1.5	10	1.308

From the data presented in the above Table 2, it is clear that 2.5 ml of BTB solution is essential to get maximum absorbance. Therefore in all further studies 2.5 ml of BTB is fixed.

Effect of concentration of drug Tamsulosin:

This study leads to the effect of the drug Tamsulosin concentration on the absorbance of Ion – Pair complex under established optimal experimental conditions. The recommended procedures for the calibration curve and for the obedience of Beer-Lambert's law for the quantitative spectrophometric determination of the drug Tamsulosin is as follows:-

Calibration Curve: Obedience of Beer-Lambert's Law:

Various aliquots (x ml i.e., 0.5 ml to 2.5 ml) of Tamsulosin solution (100 µg/ml), 1 ml of Dutasteride solution (100 µg/ml) , are taken in a series of 10 ml standard flask. To each flask, 1.0 ml of Buffer solution of pH 2.5, 2.5 ml of BTB solution (0.5% w/v), 1.5 ml of methanol followed

by distilled water are added so as to make the total volume in each case at 10 ml. The contents of each flask are shaken well and allowed to stand for a minute for equilibration. The absorbance of each solution is measured at 430 nm against a suitable reagent blank which is prepared in a similar manner but devoid of drug solution. The results obtained are mentioned in Table 3 and Figure 4 below.

x ml (0.5 ml to 2.5 ml) of Tamsulosin solution (100 µg/ml) +1 ml of Dutasteride solution (100 µg/ml) + 1.0 ml of Buffer solution of pH 2.5 + 2.5 ml of BTB solution (0.5% w/v) + 1.5 ml methanol + (3.5-x) ml distilled water = Total volume kept at 10 ml in each case. **λ_{max} = 430 nm**

Table 3: Calibration Curve – Obedience of Beer- Lambert's Law

S.No	Vol.Tamsulosin (100 µg/ml) x ml	Amount of Tamsulosin in µg/ml	Vol.of Dutasteride in ml	Vol.of Buffer Solution in ml	Vol.of BTB solution in ml	Vol.of Methanol in ml	Vol.of distilled water in ml (4-x)	Total Vol. in each flask in ml	Absorbance
1	0.5	50	1.0	1.0	2.5	2.0	3.5	10	0.321
2	1.0	100	1.0	1.0	2.5	2.0	3.0	10	0.642
3	1.5	150	1.0	1.0	2.5	2.0	2.5	10	0.896
4	2.0	200	1.0	1.0	2.5	2.0	2.0	10	1.176
5	2.5	250	1.0	1.0	2.5	2.0	1.5	10	1.486
6	3.0	300	1.0	1.0	2.5	2.0	1.0	10	1.802

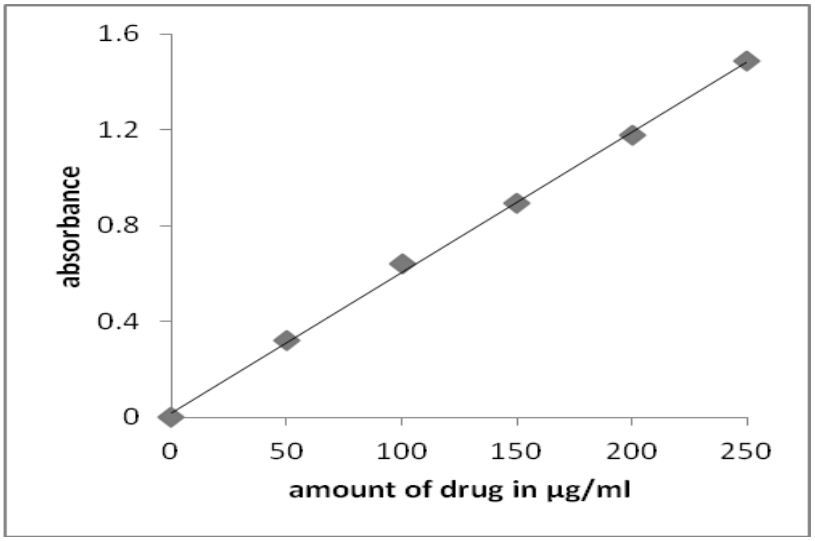

Figure 4: Calibration Curve –Verification of Beer-Lambert's Law

It is obviously clear from the data presented in the above Table 3 and from this calibration straight line (fig 4), that the absorbance values increased linearly with the increase in the amount of the drug. This verifies the Beer-Lambert's law and suggests that the method can be successfully employed for the spectrophotometric quantitative determination of the drug Tamsulosin in the

range 10 µg/ml to 300 µg/ml. The molar absorptivity and the sandell sensitivity of the method are found to be 6.6123×10^4 lit/mole/cm and 0.0067 µg/ml/cm^2 respectively.

Stoichiometric composition of Ion-Pair Complex: Job's continuous variation method:

The composition of the Ion – Pair complex between the drug Tamsulosin and the reagent BTB is established by the Job's continuous variation method. In this, the equimolar concentrations (5 x 10^{-4} M) of both the drug and BTB are varied continuously keeping the total volume of mixed solution as constant at 10 ml. In each case, the absorbance is measured at 430 nm against a suitable blank. The data obtained is presented in the below Table 4 and the Figure 5 is as shown below:

0.5 to 4.5 ml of Tamsulosin solution (5 x 10^{-4} M) +2 ml of Dutasteride solution (5 x 10^{-4} M) + 1.0 ml of Buffer solution of pH 3.5 +4.5 to 0.5 ml of BTB solution (5 x 10^{-4} M) + 2.0 ml Methanol = Total volume kept at 10 ml in each case. **λ_{max} = 430 nm**

Table 4: Job's method of continuous variation

S.No	Vol. of Tamsulosin (5 x 10^{-4}M) V_1 in ml	Vol. of Dutasteride in ml	Vol. of Buffer Solution of pH 2.5 in ml	Vol. of BTB (5 x 10^{-4} M) V_2 in ml	Vol. of Methanol in ml	Total volumein ml	Vol. fraction (x) of the drug (V_1/V_1+V_2)	Absorbance
1	0.5	2.0	1.0	4.5	2.0	10	0.1	0.489
2	1.0	2.0	1.0	4.0	2.0	10	0.2	0.882
3	1.5	2.0	1.0	3.5	2.0	10	0.3	1.253
4	2.0	2.0	1.0	3.0	2.0	10	0.4	1.670
5	2.5	2.0	1.0	2.5	2.0	10	0.5	2.158
6	3.0	2.0	1.0	2.0	2.0	10	0.6	0.803
7	3.5	2.0	1.0	1.5	2.0	10	0.7	0.571
8	4.0	2.0	1.0	1.0	2.0	10	0.8	0.483
9	4.5	2.0	1.0	0.5	2.0	10	0.9	0.369

The data in the above table are plotted in the form of a graph between volume fraction of the drug i.e., (V_1/V_1+V_2) on X- axis and the absorbance values on Y-axis. The graph obtained is as shown below in figure 5.

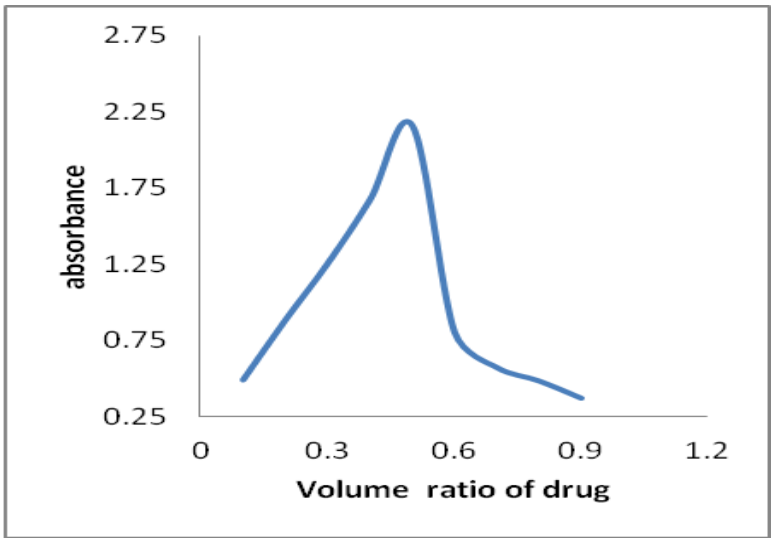

Figure 5: Job's Continuous Variation Method

From the graph shown below, it is found that one mole of the drug is reacting with 1 mole of BTB, there by establishing the stoichiometry of the Ion-Pair complex as 1:1 (Drug: BTB)

Assay of Tamsulosin drug in pharmaceutical formulations: -

The recommended procedure for the quantitative micro determination of Tamsulosin drug is applied for the assay of the drug in the dosage form of the commercial tablets and also in pharmaceutical formulations. The assay is carried out as follows:

20 tablets of Tamsulosin are weighed and finely powdered. An accurately weighed portion of the powdered sample equivalent to 50 mg of Tamsulosin is taken in a 50 ml volumetric flask containing 25 ml of methanol and is sonicated for about 20 minutes. The resultant solution is filtered through Whatman filter paper No.41 into another 50 ml volumetric flask. The filter paper is washed several times with methanol and the washings are added to filtrate. The final volume is made upto the mark with methanol. Now, 5 ml of filtrate of the sample solution is diluted to 10 ml with methanol and treated as per the recommended procedure of calibration. From this, the amount of the drug present in the sample is computed from the calibration curve. The results obtained are as shown in below Table 5.

Table 5: Assay of Tamsulosn in Tablets:

Sample	Labeled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	*t_{cal}	% RSD
Tablet I	20	20.086±0.09	100.086	2.1366	0.45
Tablet II	20	20.046±0.18	100.046	0.5714	0.90

* Average of 5 determinations based on label claim.

CONCLUSION

The calibration curve is linear up to 300 µg/ml indicating the suitability of the proposed method for the spectrophotometric determination of Tamsulosin in the range of 10 µg/ml to 300 µg/ml. The standard deviation values are found to be low showing high accuracy and reproducibility of the method. The calculated `t' values are less than the `t' theoretical values with 4 degrees of freedom at 95% level of significance. This indicates that there is no significant difference between the proposed method and the standard method. Further, there is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentration of those present in general pharmaceutical preparations. Thus the proposed method can be conveniently adopted for the routine analysis and estimation of Tamsulosin in pharmaceutical formulations.

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