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SPECTROPHOTOMETRIC OXIDATION METHOD FOR THE DETERMINATION OF TAMSULOSIN IN THE PRESENCE OF DUTASTERIDE BY USING BROMATE – BROMIDE MIXTURE

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.

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***Corresponding Author**
I. Lakshmi Prasanna
Department of Physics,
Rayalaseema University,
Kurnool.518007, AP.

ABSTRACT

A sensitive, precise, accurate, simple and rapid spectrophotometric method has been developed for the estimation of Tamsulosin in the presence of Dutasteride in pharmaceutical formulations and in the drug dosage form. During the course of study, it is observed that acidic solution of the drug formed the oxidation product with Bromate – Bromide mixture. This property of the drug is exploited for the development of spectrophotometric method for the determination and analysis of the drug. The oxidation product showed λ_{max} at 320 nm. The linearity range for Tamsulosin in the presence of Dutasteride is found to be 20 µg/ml to 300 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 evaluated and the values are found to be to be 2.0691×10^4 lit/mole/cm and 0.0215µg/ml/cm^2 respectively.

KEYWORDS: Spectrophotometry, Tamsulosin, Dutasteride, Bromate-Bromide oxidant, Pharmaceutical formulations.

INTRODUCTION

Tamsulosin (TAM), chemically 5-[(2R)-2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxybenzene-1- sulfonamide,[1-3] 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. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate. 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 the drug Tamsulosin is as shown in fig1 is as follows.

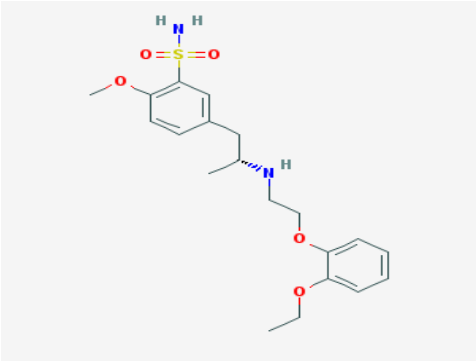

Fig 1: Structure of Tamsulosin.

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,[4-12] is a white powder and is freely soluble in acetonitrile, ethanol, methanol and partially insoluble in water. Categorized in Enzyme Inhibitors, Anti-baldness Agents, Antihyperplasia Agents. Belongs to a class of drugs called 5-alpha-reductase inhibitors, which block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone(DHT). 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 shown below in fig.2.

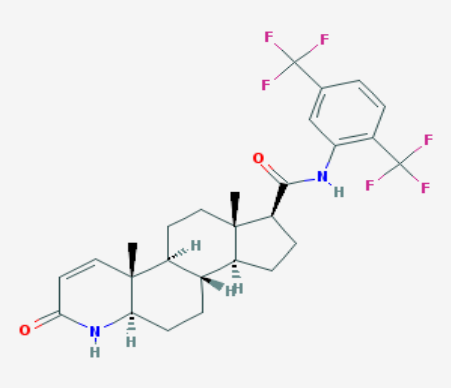

Fig 2: Structure of Dutasteride.

Combination therapy[13] 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)-adrenoceptors 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.

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 METHODS

(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.

(B) Preparation of Reagents and Solutions

(i) 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.

(ii) Dutasteride solution: 50 mg of pure Dutasteride 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.

(iii) 5M HCL solution: 425 ml of Conc Hydrochloric acid (Merck) is diluted to 1000 ml with distilled water to get about 5 M Hydrochloric Acid solution. All other chemical substances and reagents employed in the present investigations are of AR of grade only.

(iv) Bromate - Bromide Mixture preparation: 0.835 g of Potassium Bromate and 6 g of Potassium Bromide are dissolved in distilled water and diluted to one litre. The solution is appropriately diluted to get 10 µg/ml with respect to Potassium Bromate.

(v) Methyl Orange solution: 50 mg of Methyl Orange is dissolved in 50 ml water and is diluted to get 50 µg/ml. By taking 5 ml and adding 100 ml water such that the concentration becomes equal to 50 µg/ml.

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 Bromate- Bromide mixture and then Methyl Orange is added in the redox reaction. This redox reaction is spectrophotometrically monitored to develop a method for the determination of the drug. In the process of carrying out detailed investigations, first of all, optimization of various parameters such as the wavelength of maximum absorbance (λ_{max}), the effect of the concentration of oxidizing agent Bromate - Bromide mixture and the dye Methyl Orange on the absorbance of the oxidation product are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of Oxidation reaction: The absorption spectrum of the oxidation reaction between Tamsulosin in presence of Dutasteride and Bromate –Bromide mixture is obtained in order to fix the wavelength of maximum absorbance in the present study. The experimental procedure adopted is as follows:

1 ml of Tamsulosin solution (50 µg/ml), 1 ml of Dutasteride solution (50 µg/ml) ,1 ml of 5M HCl, 1 ml of Bromate-Bromide mixture, 1 ml of Methyl Orange(50 µg/ml) , are taken in a 10 ml standard flask. The resulting solution is made up to the mark with distilled water. The contents of the flask are shaken well and allowed to stand for a minute for equilibration. Then the absorbance values of the oxidation reaction formed are measured in the wavelength range 260 nm to 360 nm against the reagent blank. The results obtained are used to draw a graph between the wavelength and the absorbance values. This graphical representation is called the Absorption spectrum which is shown in figure 3 below.

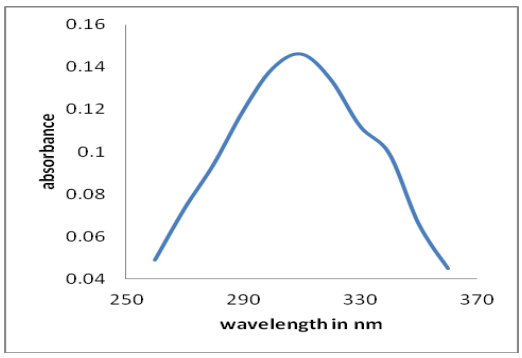

Fig 3: Absorption Spectrum of Oxidation reaction with Bromate- Bromide mixture.

It is seen from the Fig .3 of the absorption spectrum that the maximum absorbance is obtained at 320 nm. Hence for all further studies, the wavelength 320 nm is fixed.

Effect of Bromate-Bromide mixture: The effect of Bromate-Bromide mixture on the absorbance of the oxidation reaction is studied by taking varying volumes (x ml) of Bromate-Bromide mixture in a series of 10 ml standard flasks. After taking x ml (0.5 ml to 2.5 ml) of Bromate-Bromide mixture in each flask, 1ml of 5M HCl, 1 ml of drug solution of Tamsulosin,1 ml of Dutasteride solution, 1 ml of Methyl Orange are added and the resulting solution is made up to 10 ml using distilled water. The absorbance of each solution is recorded at 320 nm against a suitable blank.

Table 1: Effect of Bromate – Bromide mixture on Oxidation.

1 ml Tamsulosin solution(50 µg/ml) +1 ml of Dutasteride solution(50 µg/ml) +x ml (0.5 ml to 2.5 ml) of Bromate – Bromide mixture solution (10 µg/ml) + 1 ml of Methyl Orange + 1 ml of 5M HCl +(6-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} =320 nm.**

S. No	Vol. of Tamsulosin (50 µg/ml) in ml	Vol. of Dutasteride (50 µg/ml) in ml	Vol of 5M HCl in ml	Vol. of Bromate-Bromide mixture Solution x ml	Vol. of Methyl Orange in ml	Vol. of distilled water in ml (6-x)	Total Vol. in each flask in ml	Absorbance
1	1.0	1.0	1.0	0.5	1.0	5.5	10	0.178
2	1.0	1.0	1.0	1.0	1.0	5.0	10	0.186
3	1.0	1.0	1.0	1.5	1.0	4.5	10	0.170
4	1.0	1.0	1.0	2.0	1.0	4.0	10	0.140
5	1.0	1.0	1.0	2.5	1.0	3.5	10	0.053

It is observed that 1.0 ml of Bromate-Bromide mixture solution is required for maximum absorbance. Hence for all further studies a volume of 1.0 ml of Bromate-Bromide mixture solution is fixed.

Effect of volume of Methyl Orange: The effect of Methyl Orange on the absorbance of oxidation reaction is studied by taking varying volumes (x ml) of Methyl orange solution(50 µg/ml) in a series of 10 ml standard flasks keeping the volume of Tamsulosin solution, Dutasteride solution fixed at 1 ml. To each flask 1 ml of 5M HCl and 1.0 ml of Bromate-Bromide mixture are added followed by the addition of distilled water to make up each 10 ml flask to the mark. The absorbance of each solution is recorded at 320 nm against the suitable blank.

Table 2: Effect of Methyl Orange on Oxidation.

1 ml of Tamsulosin solution (50 µg/ml) + 1 ml of Dutasteride solution (50 µg/ml) +1 ml of 5 M HCl+ x ml (0.5 ml to 2.5 ml) of Methyl Orange solution (50 µg/ml) + 1 ml of Bromate-Bromide mixture (10 µg/ml) + (6-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 320 nm.**

S. No	Vol. of Tamsulosin (50 µg/ml) in ml	Vol. of Dutasteride in ml	Vol. of Methyl Orange solution x ml	Vol. of Bromate-Bromide mixture in ml	Vol. of 5 M HCl in ml	Vol. of distilled water in ml (6-x)	Total Vol. in each flask in ml	Absorbance
1	1.0	1.0	0.5	1.0	1.0	5.5	10	0.045
2	1.0	1.0	1.0	1.0	1.0	5.0	10	0.073
3	1.0	1.0	1.5	1.0	1.0	4.5	10	0.096
4	1.0	1.0	2.0	1.0	1.0	4.0	10	0.122
5	1.0	1.0	2.5	1.0	1.0	3.5	10	0.165
6	1.0	1.0	3.0	1.0	1.0	3.0	10	0.165

It is observed that 2.5 ml of Methyl Orange solution is necessary to achieve maximum absorbance. Hence for all further studies a volume of 2.5 ml of Methyl Orange solution is required.

Effect of concentration of Drug Tamsulosin: This study pertains to the effect of the drug Tamsulosin concentration on the absorbance of the Oxidation reaction under the established optimal experimental conditions. The recommended procedure for the calibration curve and for the obedience of Beer-Lambert's Law for the quantitative spectrophotometric 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 (50 µg/ml) , 1 ml of Dutasteride solution (50 µg/ml) are taken in a series of 10 ml standard flask. To each flask, 2.5 ml of Methyl Orange solution, 1 ml of Bromate-Bromide mixture solution, 1 ml of 5M HCl, are added followed by (4.5-x) ml of distilled water 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 320 nm against a suitable reagent blank which is prepared in a similar manner but devoid of drug solution. The results are shown in Table 3 and figure 4.

Table 3: calibration curve: - obedience of Beer-Lambert's Law.

x ml of Tamsulosin solution (50 µg/ml) + 1 ml Dutasteride solution (50 µg/ml) + 2.5 ml of Methyl Orange solution (50 µg/ml) + 1 ml of Bromate-Bromide mixture (10 µg/ml) +1 ml of 5 M HCl + (4.5-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max}= 320 nm**

S. No	Vol.of Tamsulosin (50 µg/ml) x ml	Amount of Tamsulosin in µg/ml	Vol. of Dutasteride (50 µg/ml) in ml	Vol. of Methyl Orange Solution (50 µg/ml)	Vol.of Bromate-Bromide mixture in ml	Vol of 5M HCl in ml	Vol. of distilled water in ml (4.5-x)	Total Vol. in each flask in ml	Absorbance
1	0.5	50	1.0	2.5	1.0	1.0	4.0	10	0.098
2	1.0	100	1.0	2.5	1.0	1.0	3.5	10	0.183
3	1.5	150	1.0	2.5	1.0	1.0	3.0	10	0.266
4	2.0	200	1.0	2.5	1.0	1.0	2.5	10	0.353
5	2.5	250	1.0	2.5	1.0	1.0	2.0	10	0.465
6	3.0	300	1.0	2.5	1.0	1.0	1.5	10	0.562
7	3.5	350	1.0	2.5	1.0	1.0	1.0	10	0.631

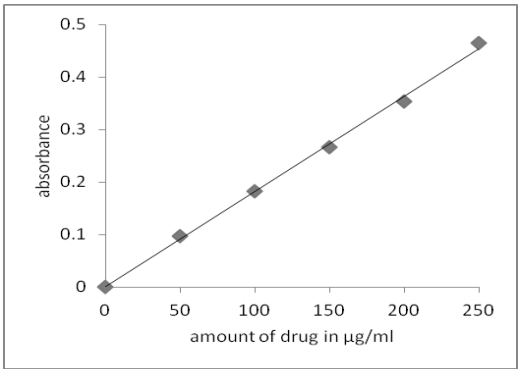

Fig. 4 : Calibration curve –Verification of Beer-Lambert's Law.

It is obviously clear from this calibration straight line as shown above in 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 20 µg/ml to 300 µg/ml. The molar absorptivity and the sandell sensitivity of the method are found to be 2.0691 $\times 10^4$ lit/mole/cm and 0.0215µg/ml/cm^2 respectively.

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 up to 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 Table 4 below.

Table 4: Assay of Tamsulosin in Tablets.

Sample	Labelled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	$^*t_{cal}$	% RSD
Tablet I	20	20.034±0.26	100.034	0.2924	1.30
Tablet II	20	20.066±0.18	100.066	0.8198	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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