SPECTROPHOTOMETRIC DETERMINATION OF TAMSULOSIN IN THE PRESENCE OF DUTASTERIDE BY CHARGE TRANSFER COMPLEX METHOD BY USING 2, 3 – DICHLORO-5, 6-DICYANO-1, 4- BENZOQUINONE (DDQ)

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***ABSTRACT*: A simple, versatile, accurate and a new spectrophotometric method is proposed for the estimation of microgram quantities of the drug Tamsulosin in the presence of Dutasteride. The drug forms a Charge Transfer (CT) complex with 2,3 –Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ), the stoichiometry of which is established as 1:1 by Job's continuous variation method. The wavelength of the maximum absorbance of the CT complex is found to be 400 nm. The absorbance values of the CT complex increased linearly with the increase in the amount of the drug Tamsulosin in the presence of Dutasteride. This suggests the suitability of the method for the determination of the drug in the range 10 µg/ml to 250 µg/ml. This also indicates the verification of the Beer-Lambert's Law in this range. The method is successfully applied to evaluate the assay of commercial tablets in pharmaceutical formulations for Tamsulosin and the results agreed very well. The molar absorptivity and Sandell Sensitivity of the method are found to be 7.0306×10^4 lit/ mole/cm and 0.0063 µg/ml/ cm^2 respectively.**

***KEYWORDS*: Spectrophotometry, Tamsulosin, Dutasteride, Charge Transfer Complex, DDQ, Pharmaceutical Formulations.**

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. 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 and is as follows:

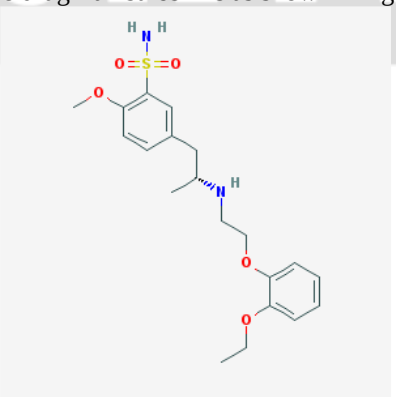

Fig 1: Structure of Tamsulosin

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,[5-13] 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.

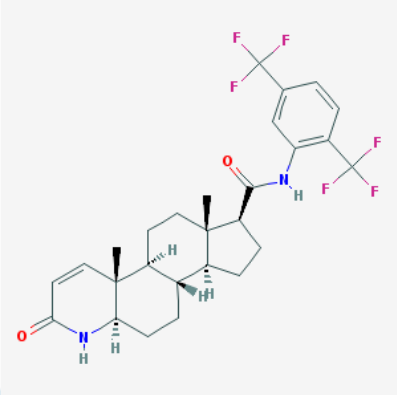

Fig 2: Structure of Dutasteride

Combination therapy 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 100 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 100 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

DDQ solution (0.1% w/v): 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone which is abbreviated as DDQ is prepared by dissolving 100 mg of it in 100 ml of Acetonitrile.

All other chemical substances and reagents employed in the present investigation are of is AR grade only.

RESULTS AND DISCUSSION:

Tamsulosin in the presence of Dutasteride when treated with 2, 3-Dichloro-5, 6-Dicyano-1, 4-Benzoquinone (DDQ) forms a charge transfer (CT) complex in which the drug Tamsulosin acts as the n - electron donor and the DDQ as the electron acceptor. This charge transfer (CT) complex formation reaction is spectrophotometrically monitored to develop the method of determination of the drug. In the absorbance (λ_{max}), the effect of concentration of DDQ on the absorbance of the charge transfer complex are established and the procedures adopted in each case are described as follows.

Absorption Spectrum of CT complex: - The absorption spectrum of the CT complex formed between Tamsulosin in the presence of Dutasteride and DDQ is obtained in order to fix the wavelength of maximum absorbance. The experimental procedure is as follows:-

1 ml of Tamsulosin solution (100 µg/ml), 1 ml of Dutasteride solution (100 µg/ml), 2 ml of DDQ solution (0.1% w/v), and 2 ml of methanol are taken in a 10 ml standard flask the resulting solution is made upto 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 CT complex formed are measured in wavelength range 320 nm to 460 nm against the reagent blank. The results are used to draw a graph between the wavelength and the absorbance values. The graph shown below in fig.3 is called the absorption spectrum.

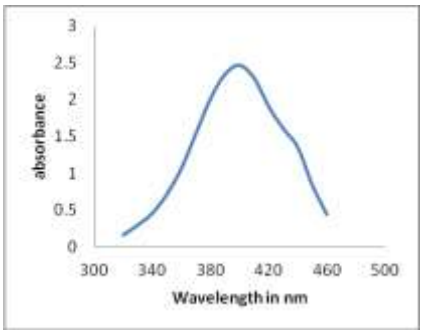

Fig.3: Absorption spectrum of CT complex of Tamsulosin with DDQ

It is seen from the above graph, that the maximum absorbance is obtained at 400 nm. Hence for all further studies the wavelength of 400 nm is fixed.

Effect of DDQ concentration:- The effect of DDQ on the absorbance of the CT complex is studied by taking varying volumes (x ml, i.e.; 0.5 ml to 2.5 ml) of DDQ in a series of 10 ml standard flasks keeping the volume of Tamsulosin solution fixed at 2 ml. To each flask 1 ml of Dutasteride solution, 2 ml of methanol is added followed by the addition of distilled water upto 10 ml in each flask. The absorbance of each solution is recorded at 400 nm against a suitable blank. The results are tabulated as below Table 1.

Table 1: Effect of DDQ on CT complex

2 ml of Tamsulosin solution (100 µg/ml) +1 ml of Dutasteride solution (100 µg/ml) +x ml of DDQ solution (0.1% w/v) + 2 ml of Methanol +(5-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 400 nm.**

S. No	Vol. of Tamsulosin in ml	Vol. of Dutaste-ride in ml	Vol. of DDQ solution x ml	Vol. of Methan-ol in ml	Vol. of distilled water in ml (5-x)	Total vol. in each flask in ml	Absorb-ance
1	2.0	1.0	0.5	2.0	4.5	10	0.299
2	2.0	1.0	1.0	2.0	4.0	10	0.414
3	2.0	1.0	1.5	2.0	3.5	10	0.688
4	2.0	1.0	2.0	2.0	3.0	10	1.070
5	2.0	1.0	2.5	2.0	2.5	10	1.176
6	2.0	1.0	3.0	2.0	2.0	10	1.176

From the data presented in table 1 above, it is clear that 2.5 ml of DDQ solution is required for maximum absorbance. Hence for all further studies a volume of 2.5 ml of DDQ solution is fixed.

Effect of concentration of drug Tamsulosin: Calibration curve: - This study pertains to the effect of the drug Tamsulosin concentration on the absorbance of the CT complex under the established optimal experimental conditions. The recommended procedure is as follows.

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 flasks. To each flask, 2.5 ml of DDQ solution (0.1% w/v), and 2 ml of methanol followed by (4.5-x) ml of distilled water are added so as to make the total volume in each case at 10 ml. The contents of each flask are made upto the mark and the absorbance of each solution is measured at 400 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 2 and fig.4 as shown below.

Table 2: Calibration curve: Applicability of Beer – Lambert's law

x ml (0.5 ml to 2.5 ml) of Tamsulosin solution (100 µg/ml) + 1 ml of Dutasteride solution (100 µg/ml) + 2.5 ml DDQ solution (0.1% w/v) + 2 ml methanol + (4.5-x) ml distilled water = Total volume kept at 10 ml in each case. **λ_{max} = 400 nm.**

S. No	Vol.of Tamsulosin (100 µg/ml) x ml	Amount of Tamsul-osin in µg/ml	Vol.of Dutaste-ride (100 µg/ml) in ml	Vol.of DDQ solution in ml	Vol.of Methanol in ml	Vol.of distilled water in ml (4.5-x)	Total Vol. of distilled water in ml	Absor-bance
1	0.5	50	1.0	2.5	2.0	4.0	10	0.4
2	1.0	100	1.0	2.5	2.0	3.5	10	0.7
3	1.5	150	1.0	2.5	2.0	3.0	10	0.9
4	2.0	200	1.0	2.5	2.0	2.5	10	1.2
5	2.5	250	1.0	2.5	2.0	2.0	10	1.48

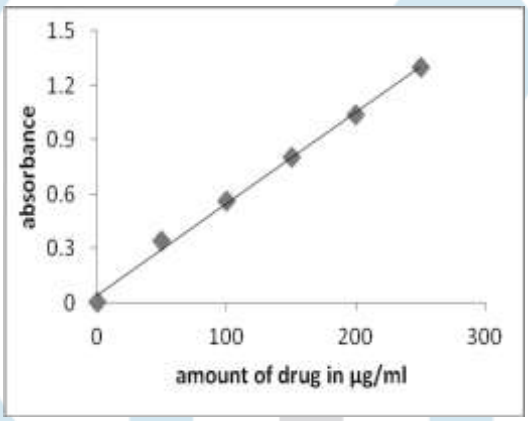

Fig.4: Calibration curve –Verification of Beer-Lambert's Law

It is obviously clear from the data presented in above Table 2 and from the calibration straight line as shown in fig.4 above 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 suitably employed for the spectrophotometric quantitative determination of the drug Tamsulosin in the range 10 µg/ml to 250 µg/ml. The molar absorptivitiy and the Sandell Sensitivity of the method are found to be 7.0306×10^4 lit/ mole/cm and 0.0063 µg/ml/ cm^2 respectively.

Stoichiometric Composition of the Charge Transfer complex : Job's Continuous Variation Method: The composition of the CT complex between the drug Tamsulosin in the presence of Dutasteride and the reagent DDQ is established by the Job's "continuous variation method". In this method, equimolar concentration (5×10^{-4} M) of both the drug and DDQ are varied continuously keeping the total volume of the mixed solution as constant at 5 ml. In each case, the absorbance is measured at 400 nm against a suitable blank. The data obtained is presented in Table 3 and Fig. 5 below.

Table 3: Job's method of continuous variation

0.5 to 4.5 ml of Tamsulosin solution (5 x 10^{-4} M) + 2 ml of Dutasteride solution (5 x 10^{-4} M) + 4.5 ml to 0.5 ml of DDQ solution (5 x 10^{-4} M) +3 ml of Methanol = Total volume kept at 10 ml in each case. **λ_{max}= 400 nm**

S. No.	Vol. of Tamsulo-sin (5 x 10^{-4} M) V_1 in ml	Vol. of Dutasteride (5 x 10^{-4} M) in ml	Vol. of DDQ (5 x 10^{-4} M) V_2 in ml	Vol. of methanol in ml	Total volume in ml	Volume fraction (X) of the drug (V_1/V_1+V_2)	Absorbance
1	0.5	2.0	4.5	3.0	10.0	0.1	0.040
2	1.0	2.0	4.0	3.0	10.0	0.2	0.095
3	1.5	2.0	3.5	3.0	10.0	0.3	0.220
4	2.0	2.0	3.0	3.0	10.0	0.4	0.270
5	2.5	2.0	2.5	3.0	10.0	0.5	0.402
6	3.0	2.0	2.0	3.0	10.0	0.6	0.150
7	3.5	2.0	1.5	3.0	10.0	0.7	0.085
8	4.0	2.0	1.0	3.0	10.0	0.8	0.069
9	4.5	2.0	0.5	3.0	10.0	0.9	0.050

The data in the above Table are plotted in the form of a graph between volume fraction ((V_1/V_1+V_2) on the x- axis and the absorbance values on the y-axis. The graph obtained is shown in figure 5 below.

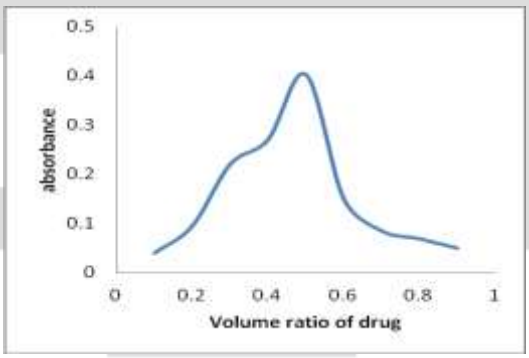

Fig.5: Job's Continuous Variation Method

From the Fig.5 shown above, it is found that one mole of the drug is reacting with one mole of DDQ thereby establishing the stoichiometry of the CT complex as 1:1 (Drug: DDQ).

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 tablet and also in the 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 the 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 shown in table 4 as shown 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	20.046±0.19	100.046	0.5413	0.95
Tablet II	20	20.040±0.16	100.040	0.5590	0.80

* 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 250 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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