

## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN IN TABLET DOSAGE FORM

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### ABSTRACT

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Dutasteride and Tamsulosin in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Acetonitrile 65:35 were set (Buffer  $P^H$  4.25 adjusted with Triethylamine), Kromasil ODS 3V (250×4.6mm, 5 $\mu$ ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 225 nm. As the methanol content was increased Dutasteride and Tamsulosin got eluted with good peak symmetric properties. The retention times for Dutasteride and Tamsulosin was found to be 3.118 min and 6.640 min, respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to 150 % levels,  $R^2$  value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Dutasteride and Tamsulosin.

### KEY WORDS

Dutasteride and Tamsulosin, RP-HPLC

### INTRODUCTION:

Dutasteride is 5-alpha-reductase inhibitors. Chemically Dutasteride, (5 $\alpha$ , 17 $\beta$ )-N-{2, 5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide.

DUTASTERIDE block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone. DHT is mainly responsible for enlargement of prostate gland<sup>(1)</sup>.

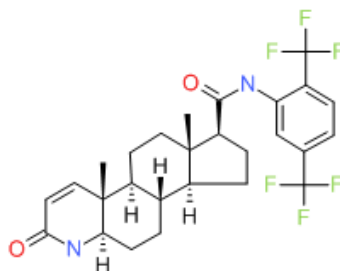


Figure 1: Structure of Dutasteride

Tamsulosin is Antineoplastic Agents, Adrenergic alpha-Antagonists. Tamsulosin, chemically it is found (5α, 17β)-N- {2,bis(trifluoromethyl) phenyl}-3-oxo-4-azandrost-1-ene-17 – carboxamide. Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate. 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, and thus decreases urinary outflow resistance in men<sup>(2)</sup>.

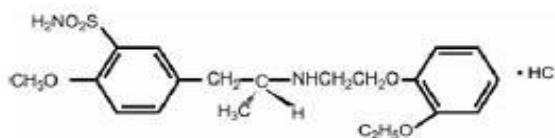


Figure 2: Structure of Tamsulosin<sup>(2)</sup>

There are very few methods reported in the literature for analysis of Dutasteride and Tamsulosin alone or in combination with other drugs in the pure form and pharmaceutical formulations by UV-Spectrophotometer, HPLC, HPTLC, LC-MS. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Dutasteride and Tamsulosin. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form.

## MATERIALS & METHODS

### Materials:

Analytically pure samples of Dutasteride & Tamsulosin were procured as gift samples from Dr. Reddy's Laboratories, (Hyderabad, India). VELTAM PLUS Tablet containing, DUTASTERIDE-0.5mg, TAMSULOSIN-0.4mg tablets manufactured by INTAS pharmaceuticals were procured from a local pharmacy. The solvents for the experiment were

selected based on the solubility test results of both the drugs. The solubility tests were performed using the common solvents like water, methanol (Sd Fine), Acetonitrile (Merck). The analytical reagents Buffer and Acetonitrile taken in the ratio 65:35 were used to prepare the mobile phase which is filtered through a nylon 0.45μm membrane filter paper.

### ASSAY METHOD DEVELOPMENT:

Different mobile phase composition and different chromatographic conditions were used in the trials in order to get well resolved peaks with good peak shapes and higher theoretical plates which pass the USP acceptance criteria of peak parameters were obtained finally.

### CHROMATOGRAPHIC CONDITIONS:

Method was developed using a Shimadzu UFLC-20AD chromatographic system (Japan), equipped with isocratic pump, and with SPD-M20A diode array detector attached with data recorder and integrator LC-10 solution software. HPLC with PDA detector (Waters); HPLC column using is Kromasil ODS 3V (250×4.6mm, 5μ) ODS C-18 RP-column; Mobile phase filtration unit was Ultipor Nylon membrane (Pall Life sciences, Mumbai, India).

**METHOD VALIDATION:** The developed RP-HPLC method for simultaneous estimation of Dutasteride & Tamsulosin formulation was validated as per ICH guidelines.

### ASSAY OF FORMULATION:

#### Preparation of Standard solution:

Accurately Weighed and transferred 0.4mg of Tamsulosin and 0.5mg of Dutasteride working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents (Stock Solution). From stock solution Dutasteride 2.5 ml and Tamsulosin 2.5ml was taken in 10 ml volumetric flask and volume was made up to the mark with diluent (Standard Solution). In this Dutasteride contains 12.5( $\mu\text{g/ml}$ ) and Tamsulosin 10( $\mu\text{g/ml}$ ).

#### Sample Preparation:

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 10 mL volumetric flask, 3mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 2.5ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent. In this sample preparation containing 25  $\mu\text{g/ml}$ .

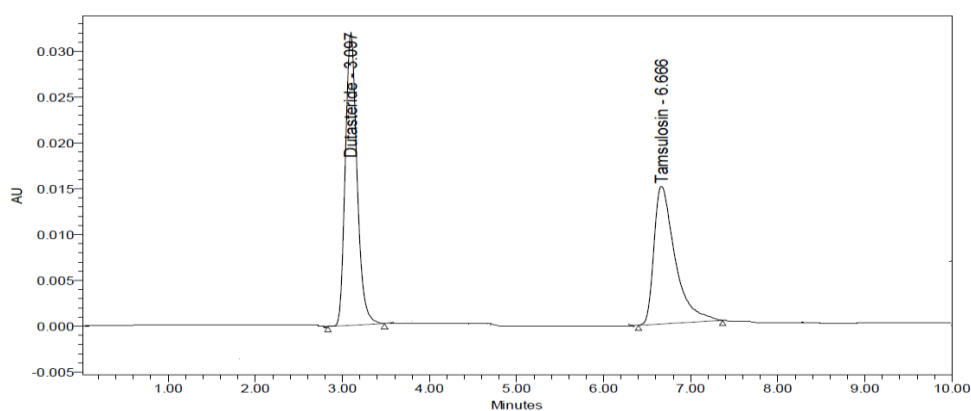


Figure 3: Chromatogram of Standard

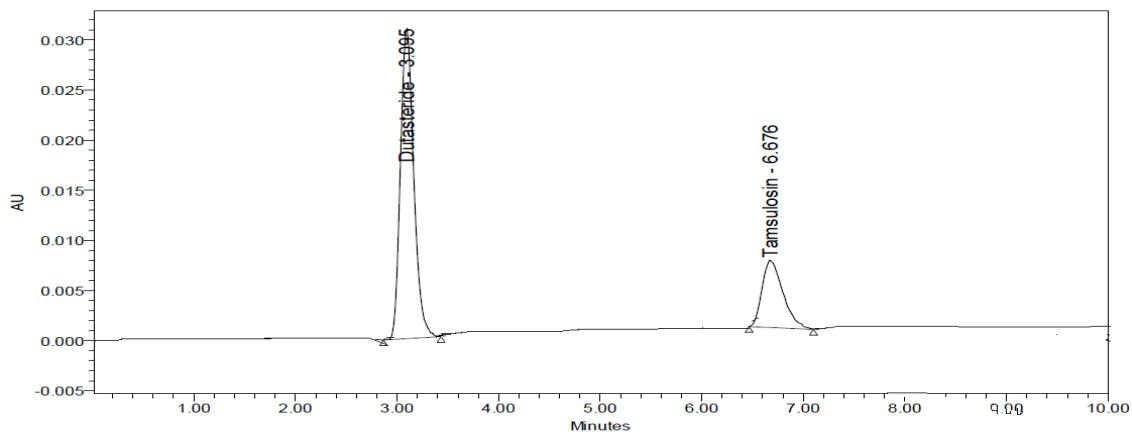


Figure 4- Chromatogram of Test

**Table 1: Peak results of Standard & Test Chromatograms for Assay**

| Parameter       | Standard    |            | Test        |            |
|-----------------|-------------|------------|-------------|------------|
|                 | Dutasteride | Tamsulosin | Dutasteride | Tamsulosin |
| Retention time  | 3.097       | 6.666      | 3.095       | 6.676      |
| Peak Area       | 345253      | 154382     | 356194      | 155799     |
| USP Plate Count | 2633        | 4837       | 2521        | 4645       |
| Tailing Factor  | 1.20        | 1.41       | 1.21        | 1.41       |

**Table 2: Results of Assay**

| Parameters            | Dutasteride | Tamsulosin |
|-----------------------|-------------|------------|
| Standard peak area    | 345253      | 154382     |
| Test peak area (mean) | 356194      | 155799     |
| Average Weight        | 5mg         | 5mg        |
| Label claim           | 0.5mg       | 0.4mg      |
| % Purity of Standard  | 99.50       | 99.58      |
| Amt obtained          | 0.48mg      | 0.36mg     |
| % Assay               | 101.5%      | 100.75%    |

The % assays of Dutasteride and Tamsulosin were found to be 101.5% and 100.75% respectively. Thus, % Assay results were found to be within the limits i.e., 98-102% for both the drugs. Hence the developed method can be routinely used for the simultaneous estimation of Dutasteride and Tamsulosin in the marketed formulations.

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters. After development of RP-HPLC method for estimation of Dutasteride and Tamsulosin, validation of the method was carried out according to ICH guidelines

The developed method was validated for the following parameters.

- System suitability
- Linearity
- Specificity
- Precision
- Accuracy

F. LOD & LOQ

G. Robustness

#### SYSTEM SUITABILITY TEST (SST)

A Standard solution of Dutasteride and Tamsulosin working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.

#### LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Serial dilutions of Dutasteride and Tamsulosin (5-15µg/ml) were injected into the column and detected at a wavelength set at 225nm. The calibration curve was obtained by plotting the concentration vs. peak area. Concentration range 5-15µg/ml was found to be linear with  $r^2=0.9986$ .

**Table 3: Preparation of Working standard solutions for Linearity**

| Working standard solutions (Level in %) | Stock solution taken in (ml) | Stock Solution taken in (ml) | Diluted to volume (ml) with diluent | Concentration of Dutasteride (µg/ml) | Concentration of Tamsulosin (µg/ml) |
|-----------------------------------------|------------------------------|------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|
| 50%                                     | 1.25                         | 1.25                         | 10                                  | 5                                    | 5                                   |
| 75%                                     | 1.875                        | 1.875                        | 10                                  | 7.5                                  | 7.5                                 |
| 100%                                    | 2.5                          | 2.5                          | 10                                  | 10                                   | 10                                  |
| 125%                                    | 3.125                        | 3.125                        | 10                                  | 12.5                                 | 12.5                                |
| 150%                                    | 3.75                         | 3.75                         | 10                                  | 15                                   | 15                                  |

#### SPECIFICITY:

ICH defines specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

#### PRECISION:

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported.

The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

#### METHOD PRECISION:

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Dutasteride and Tamsulosin formulation. The % RSD of the assay value for six determinations should not be more than 2.0%.

#### SYSTEM PRECISION:

System precision was established to ensure that the optimized analytical method is precise. System

precision was performed by injecting six replicate injections of standard solution at 100% concentration and the chromatograms were reviewed for the %RSD of peak areas. % RSD of the assay value for six determinations should not be more than 2.0%.

#### ACCURACY:

Accuracy of the method was determined by recovery experiments. There are mainly 2 types of recovery studies are there.

- Standard addition method:* To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
- Percentage method:* For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

*Acceptance criteria:* The mean % recovery of the Dutasteride and Tamsulosin at each level should be not less than 95.0% and not more than 105.0%.

The Sensitivity of measurement of Dutasteride and Tamsulosin by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where,  $\sigma$  is the standard deviation of intercept of calibration plot and  $S$  is the average of the slope of the corresponding calibration plot. The LOD and LOQ values for Dutasteride and Tamsulosin were reported in the Table.

**Table 4: LOD and LOQ Data of Dutasteride and Tamsulosin**

| Dutasteride         |                   |                                                                | Tamsulosin          |                   |                                                                  |
|---------------------|-------------------|----------------------------------------------------------------|---------------------|-------------------|------------------------------------------------------------------|
| Conc.(x)<br>(µg/ml) | Peak Areas<br>(y) | Statistical Analysis                                           | Conc.(x)<br>(µg/ml) | Peak Areas<br>(y) | Statistical Analysis                                             |
| 5                   | 154331            | S = 91445<br>c = 70793<br><br>LOD: 2.55µg/ml<br>LOQ: 7.44µg/ml | 5                   | 80933             | S = 16099<br>c = 725.8<br><br>LOD: 0.148 µg/ml<br>LOQ: 0.45µg/ml |
| 10                  | 345391            |                                                                | 10                  | 162428            |                                                                  |

#### ROBUSTNESS:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the Flow rate was made to evaluate the impact on the method.

#### Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard and Test solutions of 100% concentration was prepared & injected into the HPLC system by keeping flow rates 0.9 ml/min & 1.1 ml/min. The effect of variation of flow rate was evaluated.

#### Effect of variation in mobile phase composition:

A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of organic solvent i.e., Buffer: Acetonitrile by  $\pm 2$ ml. Standard & test solutions of 100% concentration were prepared and injected into the HPLC system and the chromatograms were recorded. The retention times, tailing factors & %RSD values were calculated.

## RESULTS AND DISCUSSION

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds.

Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of orthophosphoric acid buffer solution: Acetonitrile (65:35v/v, pH 2.45) with a flow rate of 1.0 ml/min is quite robust.

The optimum wavelength for detection was 225 nm at which better detector response for both the drugs was obtained. The retention times for Dutasteride and Tamsulosin was found to be  $3.118 \pm 0.005$  min and  $6.640 \pm 0.009$  min, respectively. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 5 to 15 µg/ml and 5 to 15 µg/ml, with regression 0.9979 and 0.9999, Dutasteride and Tamsulosin respectively. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found above 99.3 % for both the drugs.

Robustness of the proposed method was determined by varying various parameters, the %RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

# SYSTEM SUITABILITY:

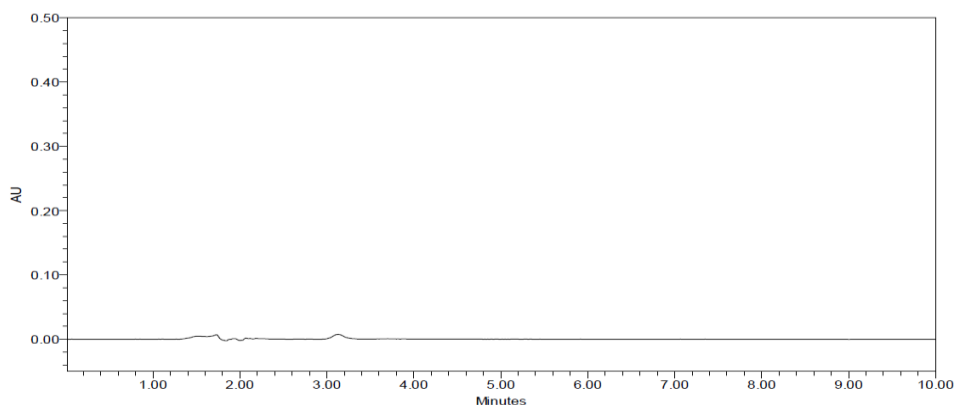


Figure 5: Chromatogram of Blank

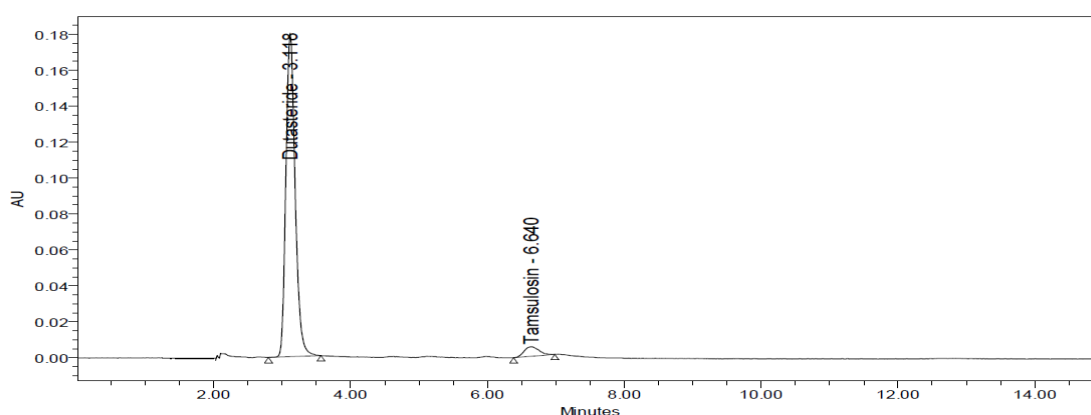


Figure 6: Chromatogram of system suitability

Table 5: Results of System suitability Test for TAMSULOSIN

| Injection | Retention time ( $t_R$ ) | Peak Area | Plate count | Tailingfactor |
|-----------|--------------------------|-----------|-------------|---------------|
| 1         | 6.666                    | 154507    | 4891        | 1.37          |
| 2         | 6.668                    | 154080    | 4334        | 1.88          |
| 3         | 6.675                    | 154111    | 4668        | 1.41          |
| 4         | 6.709                    | 154801    | 4253        | 2.20          |
| 5         | 6.712                    | 154108    | 4837        | 1.30          |
| 6         | 6.714                    | 154682    | 4320        | 1.99          |
| Mean      | -                        | 154382    | -           | -             |
| SD        | -                        | 322.7     | -           | -             |
| % RSD     | -                        | 0.2       | -           | -             |

Table 6: Results of System suitability Test for DUTASTERIDE

| Injection | Retention time (t <sub>R</sub> ) | Peak Area | Plate count | Tailing Factor |
|-----------|----------------------------------|-----------|-------------|----------------|
| 1         | 3.100                            | 348066    | 2607        | 1.22           |
| 2         | 3.110                            | 346100    | 2553        | 1.21           |
| 3         | 3.110                            | 349660    | 2565        | 1.19           |
| 4         | 3.112                            | 349065    | 2577        | 1.22           |
| 5         | 3.114                            | 346976    | 2633        | 1.20           |
| 6         | 3.116                            | 331653    | 2642        | 1.21           |
| Mean      | -                                | 345253    | -           | -              |
| SD        | -                                | 1462.0    | -           | -              |
| % RSD     | -                                | 0.4       | -           | -              |

#### LINEARITY GRAPH

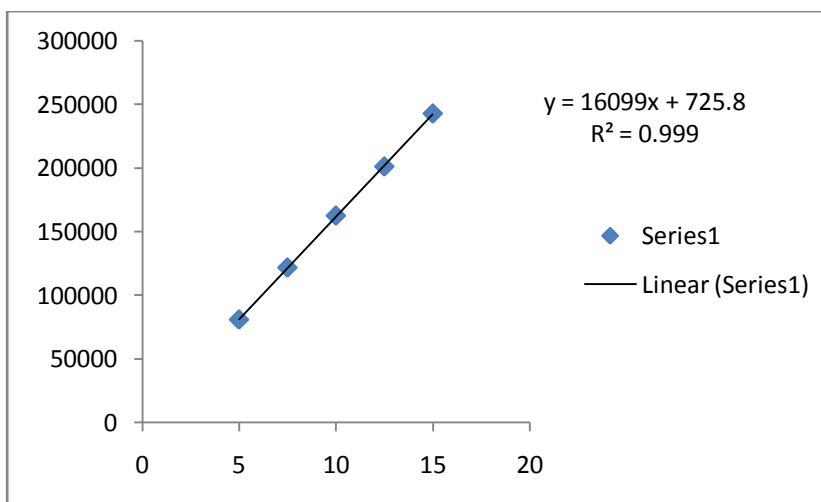


Figure 7: Linearity Graph of TAMSULOSIN

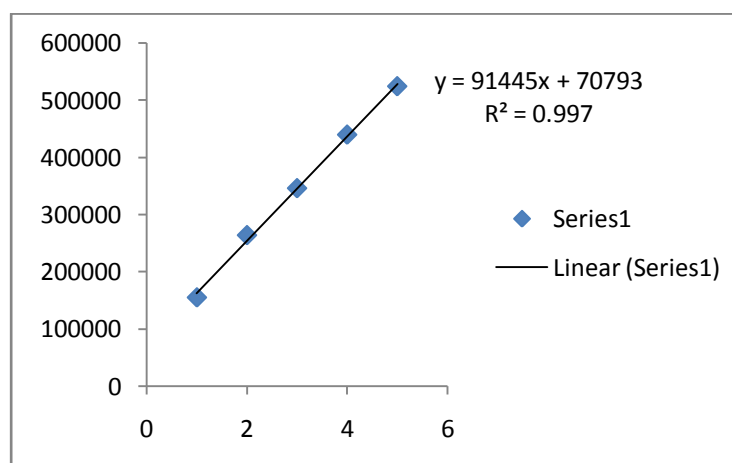


Figure 8: Linearity Graph of DUTASTERIDE

## SPECIFICITY

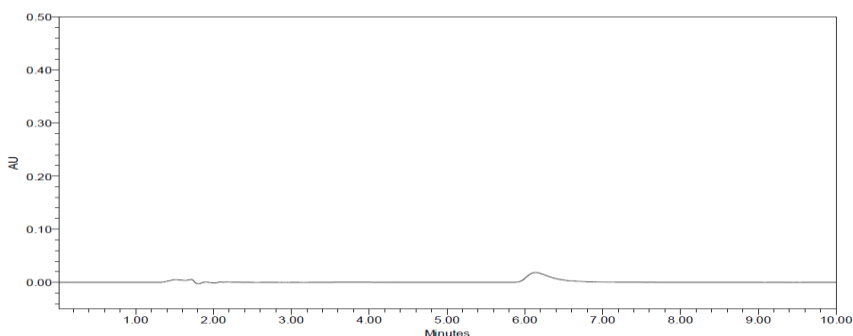


Figure 9:Chromatogram of Blank

## METHOD PRECISION

Table 7: Method Precision data for Dutasteride & Tamsulosin

| S.No. | Concentration<br>(µg/ml) | Dutasteride           |          | Tamsulosin            |           |
|-------|--------------------------|-----------------------|----------|-----------------------|-----------|
|       |                          | Retention<br>time(Rt) | PeakArea | Retention<br>time(Rt) | Peak Area |
| 1     | 10                       | 3.093                 | 343058   | 6.655                 | 153942    |
| 2     | 10                       | 3.094                 | 342802   | 6.661                 | 153336    |
| 3     | 10                       | 3.095                 | 343849   | 6.672                 | 153546    |
| 4     | 10                       | 3.099                 | 343338   | 6.675                 | 154916    |
| 5     | 10                       | 3.100                 | 344509   | 6.675                 | 154880    |
| 6     | 10                       | 3.107                 | 347256   | 6.675                 | 153281    |
| Avg   |                          |                       | 344135   |                       | 153984    |
| SD    |                          |                       | 679.7    |                       | 745.5     |
| %RSD  |                          |                       | 0.2      |                       | 0.4       |

Table 8: Intermediate Precision data for Dutasteride and Tamsulosin

| S.No. | Concentration<br>(µg/ml) | Intermediate Precision |              |                     |              |
|-------|--------------------------|------------------------|--------------|---------------------|--------------|
|       |                          | Day 1<br>Dutasteride   |              | Day 1<br>Tamsulosin |              |
|       |                          | Retention<br>time      | Peak<br>Area | Retention<br>time   | Peak<br>Area |
| 1     | 10                       | 3.063                  | 343552       | 6.636               | 176310       |
| 2     | 10                       | 3.066                  | 338959       | 6.641               | 176473       |
| 3     | 10                       | 3.066                  | 341762       | 6.643               | 174424       |
| 4     | 10                       | 3.067                  | 339609       | 6.645               | 173949       |
| 5     | 10                       | 3.076                  | 334927       | 6.650               | 173866       |
| 6     | 10                       | 3.078                  | 338481       | 6.657               | 173284       |
| Avg   |                          |                        | 339548       |                     | 174718       |
| SD    |                          |                        | 2959.0       |                     | 1347.1       |
| %RSD  |                          |                        | 0.9          |                     | 0.7          |

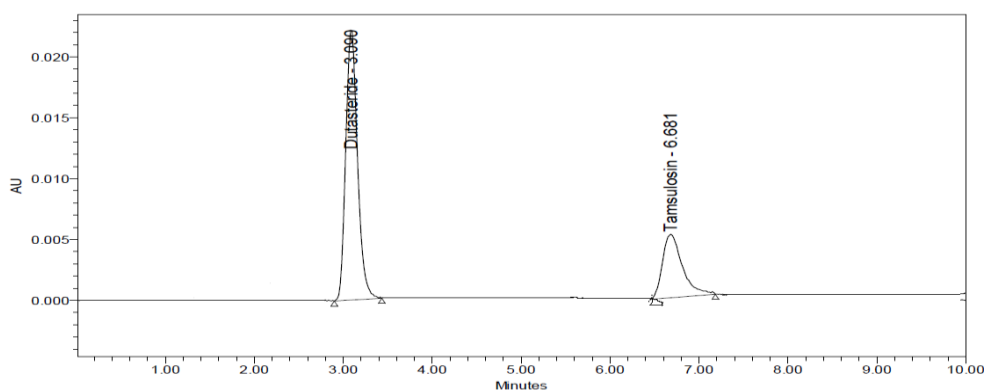
**Table 9: Intermediate Precision data for Dutasteride and Tamsulosin**

| S.No. | Concentration<br>(µg/ml) | Intermediate Precision |              |                     |              |
|-------|--------------------------|------------------------|--------------|---------------------|--------------|
|       |                          | Day 2<br>Dutasteride   |              | Day 2<br>Tamsulosin |              |
|       |                          | Retention<br>time      | Peak<br>Area | Retention<br>time   | Peak<br>Area |
| 1     | 10                       | 3.043                  | 343342       | 6.631               | 176348       |
| 2     | 10                       | 3.052                  | 335959       | 6.655               | 176424       |
| 3     | 10                       | 3.061                  | 345712       | 6.644               | 174424       |
| 4     | 10                       | 3.066                  | 339562       | 6.651               | 173985       |
| 5     | 10                       | 3.071                  | 334986       | 6.654               | 173745       |
| 6     | 10                       | 3.076                  | 336794       | 6.660               | 173755       |
| Avg   |                          |                        | 337647       |                     | 174568       |
| SD    |                          |                        | 2946.0       |                     | 1341.6       |
| %RSD  |                          |                        | 0.9          |                     | 0.8          |

**Table 10: System Precision data for Dutasteride & Tamsulosin**

| S.No. | Dutasteride           |        | Tamsulosin            |        |
|-------|-----------------------|--------|-----------------------|--------|
|       | Retention<br>time(Rt) | Area   | Retention<br>time(Rt) | Area   |
| 1     | 3.062                 | 391568 | 6.627                 | 174672 |
| 2     | 3.066                 | 387880 | 6.629                 | 175847 |
| 3     | 3.068                 | 392046 | 6.652                 | 174040 |
| 4     | 3.069                 | 391263 | 6.653                 | 178138 |
| 5     | 3.069                 | 386287 | 6.654                 | 174511 |
| 6     | 3.070                 | 385296 | 6.659                 | 17588  |
| Avg   |                       | 389057 |                       | 175633 |
| SD    |                       | 294301 |                       | 1546.2 |
| %RSD  |                       | 0.8    |                       | 0.8    |

#### ACCURACY



**Figure 10: Chromatogram of Accuracy 50%**

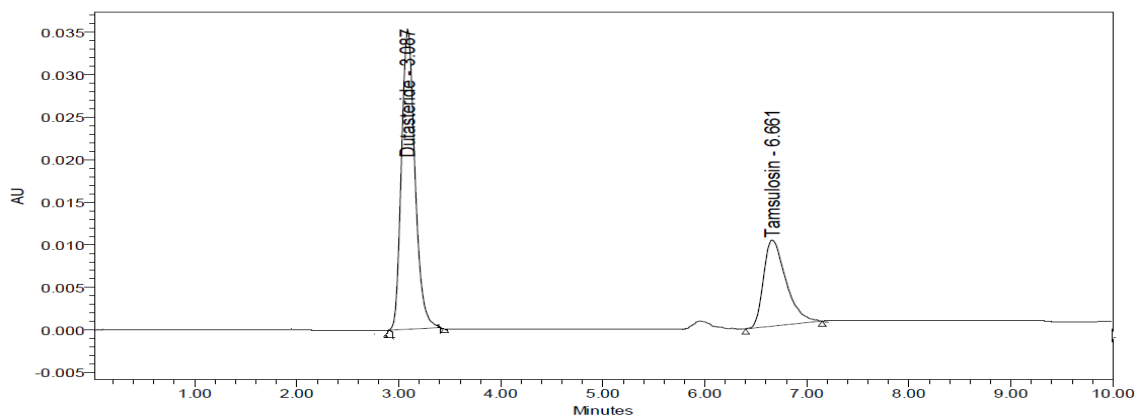


Figure 11: Chromatogram of Accuracy 100%

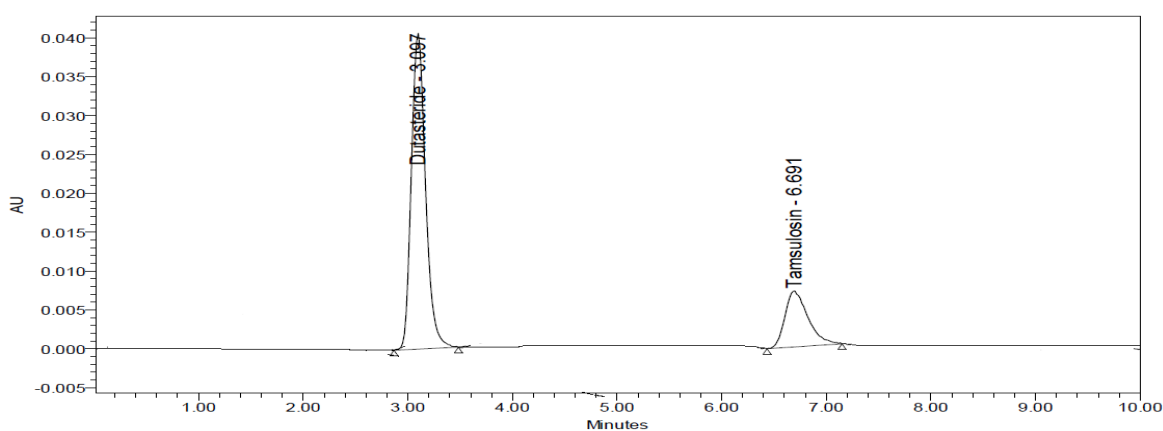


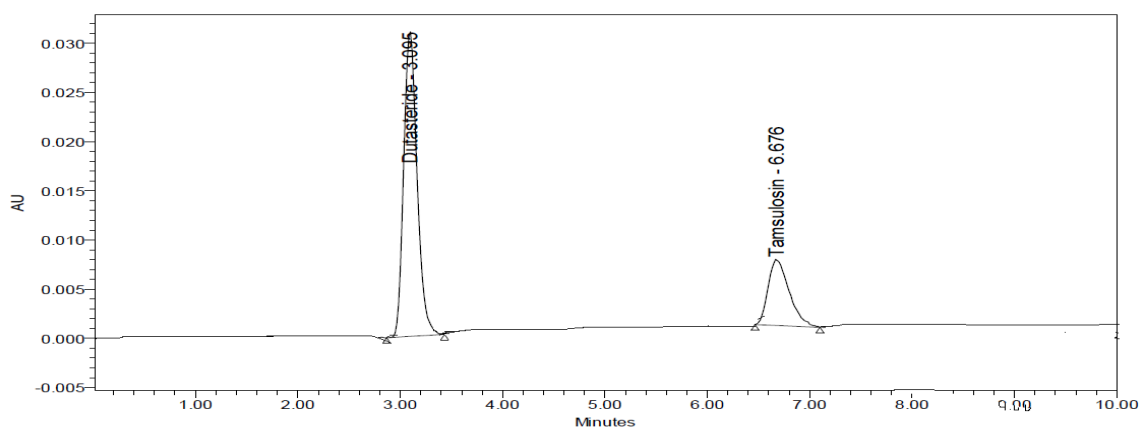
Figure 12:Chromatogram of Accuracy 150%

Table 11: Accuracy Study of Dutasteride

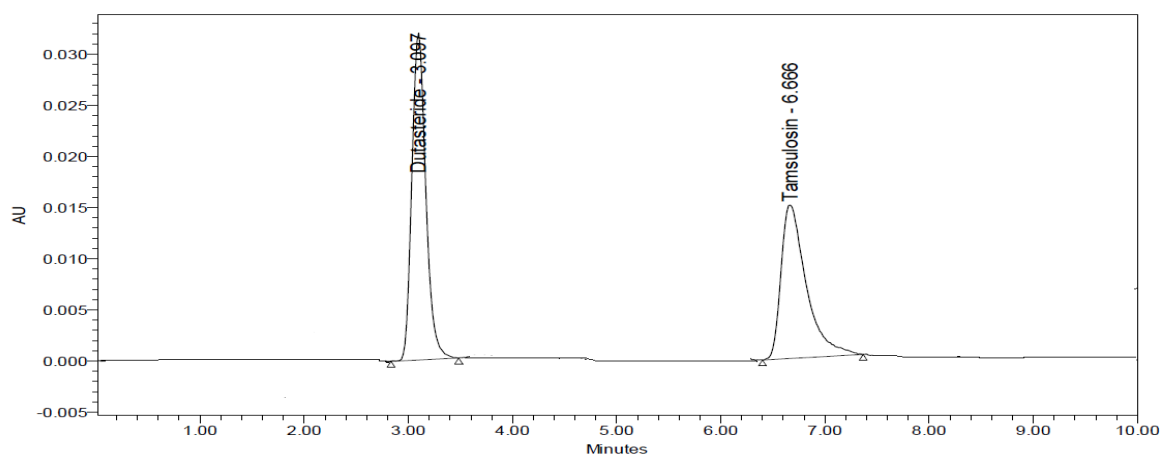
| Sample Id | Conc found (µg/ml) | Concn Obtained (µg/ml) | %Recovery | Mean recovery | Statistical Analysis |
|-----------|--------------------|------------------------|-----------|---------------|----------------------|
| 50%       | 5                  | 4.86                   | 99.4      |               | %RSD= 0.293          |
| 50%       | 5                  | 4.87                   | 99.99     | 99.6          |                      |
| 50%       | 5                  | 4.82                   | 99.54     |               |                      |
| 100%      | 10                 | 9.68                   | 100.6     |               | %RSD=0.84            |
| 100%      | 10                 | 9.72                   | 99.25     | 100.2         |                      |
| 100%      | 10                 | 9.8                    | 100.75    |               |                      |
| 150%      | 15                 | 14.7                   | 101.806   |               | %RSD=1.14            |
| 150%      | 15                 | 14.81                  | 101.313   | 100.5         |                      |
| 150%      | 15                 | 14.89                  | 99.605    |               |                      |

**Table 12: Accuracy Study of Tamsulosin**

| Conc (µg/ml) | Concn Obtained(µg/ml) | %Recovery of drug | Mean accuracy | %RSD  |
|--------------|-----------------------|-------------------|---------------|-------|
| 5            | 4.6                   | 99.308            | 99.41         | 0.18  |
| 5            | 4.7                   | 99.621            |               |       |
| 5            | 4.5                   | 99.321            |               |       |
| 10           | 9.3                   | 100.29            | 99.69         | 0.562 |
| 10           | 9.4                   | 99.460            |               |       |
| 10           | 9.6                   | 99.324            |               |       |
| 15           | 14.2                  | 100.998           | 100.52        | 0.530 |
| 15           | 14.6                  | 100.691           |               |       |
| 15           | 14.9                  | 99.960            |               |       |



**Figure 13: Representative Chromatogram at Flow rate of 0.9 ml/min**



**Figure 14: Representative Chromatogram at Flow rate of 1.1 ml/min**

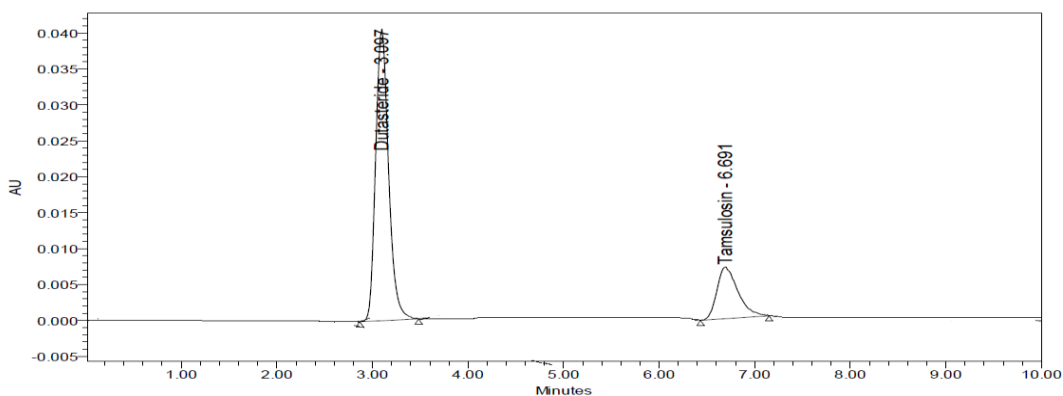


Figure 15: Representative Chromatogram for Mobile phase composition  
(Buffer: Acetonitrile::67:33)

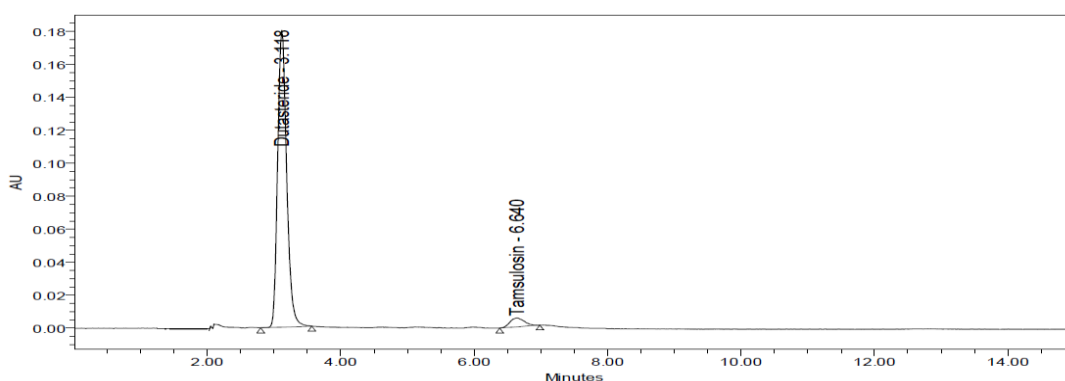


Figure 16: Representative Chromatogram for Mobile phase composition (Buffer: Acetonitrile: 63:37)

Table 13: Robustness data for Dutasteride

| Std. Replicate     | Variation in flow rate |                        | Variation in Mobile phase composition |                                 |
|--------------------|------------------------|------------------------|---------------------------------------|---------------------------------|
|                    | Flow Rate<br>0.9ml/min | Flow Rate<br>1.1ml/min | Buffer: Acetonitrile<br>(67:33)       | Buffer: Acetonitrile<br>(63:37) |
| 1                  | 442083                 | 331355                 | 362871                                | 339895                          |
| 2                  | 443968                 | 334083                 | 369307                                | 338125                          |
| Mean               | 443026                 | 332719                 | 366089                                | 339010                          |
| SD                 | 1332.9                 | 1929.0                 | 4551.1                                | 1251.8                          |
| %RSD               | 0.3                    | 0.6                    | 1.2                                   | 0.4                             |
| Retention time     | 3.428                  | 3.451                  | 3.691                                 | 3.001                           |
| Tailing factor     | 1.17                   | 1.21                   | 1.21                                  | 1.21                            |
| Theoretical plates | 2476                   | 2594                   | 2645                                  | 2624                            |

**Table 14: Robustness data for Tamsulosin**

| Parameter          | Variation in flow rate |                        | Variation in Mobile phase composition |                                 |
|--------------------|------------------------|------------------------|---------------------------------------|---------------------------------|
|                    | Flow Rate<br>0.9ml/min | Flow Rate<br>1.1ml/min | Buffer: Acetonitrile<br>(67:33)       | Buffer: Acetonitrile<br>(63:37) |
| 1                  | 134400                 | 117576                 | 155130                                | 161083                          |
| 2                  | 133624                 | 116287                 | 156599                                | 158101                          |
| Mean               | 134012                 | 116932                 | 155865                                | 159592                          |
| SD                 | 548.2                  | 911.5                  | 1038.7                                | 2108.4                          |
| %RSD               | 0.4                    | 0.8                    | 0.7                                   | 1.3                             |
| Retention time     | 7.437                  | 7.478                  | 6.349                                 | 6.397                           |
| Tailing factor     | 1.22                   | 1.19                   | 1.38                                  | 1.46                            |
| Theoretical plates | 4804                   | 4962                   | 4505                                  | 4499                            |

### CONCLUSION

A simple, specific, precise, accurate, rapid and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Dutasteride and Tamsulosin in tablet dosage form. The method was successfully validated in terms of linearity, precision, accuracy & robustness, LOD, LOQ as per ICH guidelines.

System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to 150 % levels,  $R^2$  value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present.

Full length method was not performed; if it is done this method can be used for routine analysis of Dutasteride and Tamsulosin.

### ACKNOWLEDGEMENT

The authors are very thank full to our most respected correspondent Sri Shivani College of Pharmacy, and Affiliated to Kakatiya University, Warangal, Andhra Pradesh, India for providing necessary facilities in the college campus to carry out this dissertation work successfully.

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