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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DUTASTERIDE WITH TAMSULOSIN IN PHARMACEUTICAL CAPSULE DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

Analytical method development & validation play important roles in the discovery development & manufacture of pharmaceuticals. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. A mixture of buffer solution and methanol in the ratio of (45:55). Drugs showed maximum absorbance at 280 nm for Dutasteriod and 225 nm for Tamsulosin. The % Accuracy is within limit (98.0 – 102.0 %) with %RSD less than 2%. The Precision RSD of Assay of six sample preparations was found to be 2.0%. The correlation coefficient value should not be less than 0.995 over the working range. The validation was performed as per International Conference on Harmonization (ICH) guidelines.

KEYWORDS: Dutasteriod, Tamsulosin, Reversed phase HPLC (RP-HPLC).

INTRODUCTION

Benign prostatic hyperplasia (BPH), also called benign enlargement of the prostate (BEP), adenofibromyomatous hyperplasia and benign prostatic hypertrophy, is an increase in size of the prostate. BPH involves hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of large, fairly discrete nodules in the periurethral region of the prostate. When sufficiently large, the nodules compress the urethral canal to cause partial, or sometimes virtually complete, obstruction of the urethra, which interferes with the normal flow of urine[(1).] It leads to symptoms of urinary hesitancy, frequent urination, dysuria (painful urination), increased risk of urinary tract infections, and urinary retention. Although prostate specific antigenlevels may be elevated in these patients because of increased organ volume and inflammation due to urinary tract infections, BPH does not lead to cancer or increase the risk of cancer. Combination Therapy with Dutasteride and Tamsulosin: Treatment of Benign Prostatic Hyperplasia. Pharmacological treatment of symptomatic benign prostatic hyperplasia (BPH) has become a fairly established modality [(4).] Approaches include blockade of α-adrenoreceptors and suppression of androgens. Patients eligible for drug treatment are those with mild to moderate symptoms of BPH and no strong indications for surgery. α-receptor blockers generally improve urinary symptoms and peak urinary flow rates 2 to 4 weeks after introduction of therapy. Because of minor adverse effects, selective α1-blockers are preferred over nonselective drugs[(2).] Tamsulosin, Prazosin, Terazosin and Alfuzosin are extensively studied and widely used in BPH treatment. 5-aredctase (5AR) therapy induces reduction in prostate volume (PV)

and relief in symptoms of bladder outlet obstruction. However, the only two drugs which seem to be of major interest in BPH treatment are Dutasteride and Finasteride. Future approaches in medical treatment of BPH might be combination therapy of α1-blockers and 5 α-reductase.[3]

DUTASTERIDE: [5,6,7]

Dutasteride is a dual 5-α-reductase inhibitor that inhibits conversion of testosteron to conversion dihydrotestosterone (DHT).Dutasteride is approved by the Food and Drug Administration (FDA) for treatment of benign prostatic hyperplasia (BPH) and prescribed off-label for treatment of male pattern baldness(MPB).

Description of drug

CHEMICAL NAME- (5α, 17β)-*N*-{2,5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide.

EMPIRICAL FORMULA- $C_{27}H_{30}F_6H_2O_2$

TAMSULOSIN:

Tamsulosin is a selective α_1 receptor antagonist that has preferential selectivity for theα_{1A} receptor in the prostate versus the α_{1B} receptor in the blood vessels.[5]When alpha 1 receptors in the bladder neck and the prostate are blocked, this causes a relaxation in smooth muscle and therefore less resistance to urinary flow. Due to this the pain associated with BPH can be reduced.

Description of drug

CHEMICAL NAME- (*R*)-5-(2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl)-2-methoxybenzene-1-sulfonamide.

EMPIRICAL FORMULA- $C_{20}H_{28}N_2O_5S$

MATERIALS AND METHODS

Dutasteride (DUTA) and TAMSULOSIN (TAMSU) supplied by Zydus Cadila Healthcare.

ESTIMATED METHODOLOGY FOR DUTASTERIDE:

Mobile phase preparation: 90 parts of Acetonitrile (HPLC grade) and 10 part of water (HPLC grade) was mixed well. And sonicate for 15min to remove the gases impurity. Then filter the mobile by using filtration assembly containing 0.45µ pore filter paper to remove small particals.

Preparation of Diluent: Prepared a mixture of Acetonitrile: water in the ratio of 90: 10 which was used as diluents for dilution of standard stock solution.

Stock solution of DUTA (1000 µg/mL): An accurately weighed quantity of Dutasteride working/reference standard about 50 mg was transferred into 50 mL volumetric flask and dissolve in diluents and diluted up to the mark with diluent to give a stock solution having strength 1mg/ml (1000 µg/ml).

Working standard solution of DUTA: Accurately pipette out 5 ml of dutasteride stock solution into 50ml volumetric flask and diluted up to mark with diluent. Shake wellgive a solution having strength 0.1mg/ml (100 µg/ml) (DUTA)

Sample preparation (100 µg/mL): 20 capsules were accurately weighted and pith capsules. The oil from the 20 capsules were drawn out. The empty shells of 20 Capsules were dipped into the Chloroform for 10 min. After that shells of capsules were dried at room temperature. The weight of empty shells taken for the net content of oil. The each capsule contain 350mg of oil in which 0.5mg of dutasteride. The weight of oil equivalent to 2.5mg of dutasteride was taken into 25ml of volumetric flask. Volume makes up to mark with diluents. So that concentration of dutasteride was 100µg/ml obtained.

ESTIMATED METHODOLOGY FOR TAMSULOSIN:

Buffer preparation: Dissolve 1.277g of disodium hydrogen phosphate anhydrous (Na_2HPO_4) and 0.136 g potassium dihydrogen orthophosphate (KH_2PO_4) in about 500 ml of milliQ water. Adjust the pH 7.0 using dilute solution of orthophosphate acid and mix. Filter the solution through 0.45 µm membrane filter.

Mobile phase preparation: Prepare a degassed mixture of buffer solution and methanol in the ratio of (45:55)

Preparation of Diluent: Prepared a mixture of Acetonitrile: water in the ratio of 90: 10 which was used as diluents for dilution of standard stock solution.

Standard solution (8 µg/mL): Transfer an accurately weight quantity of about 40 mg of Tamsulosin Hydrochloride working standard to a 250 ml volumetric flask. Add about 100 ml of methanol and sonicate to dissolve.

Make volume up to the mark with methanol and mix. Dilute 5 ml of this solution to 100 ml with mobile phase and mix.

Sample preparation (8 µg/mL): Weigh accurately counted 20 capsules. Open the capsules and remove pellets. Again weigh the capsules (with soft gelatin capsules). Calculate the average net content of pellets. Transfer an accurately weight pellets equivalent to 2 mg of Tamsulosin Hydrochloride to a 250 ml of volumetric flask. Add 30 ml of 1 N sodium hydroxide and sonicate for 30 minutes. Add 70 ml of methanol and further sonicate for 15 min. make volume up to the mark with mobile phase and mix. Filter the solution through 0.45 µm Millipore PVDF filter, collect the filtrate by discarding first 5 ml of the filtrate.

RESULT AND DISCUSSION

HPLC method development, optimization and validation of Dutasteride

Selection of detection wavelength: Drugs showed maximum absorbance at 280 nm for Dutasteriod and 225 nm for Tamsulosin.

Selection of mobile phase: Dutasteride is freely soluble in acetonitrile. acetonitrile was used to optimize the retention time of late eluting drug. Mobile phase ACN:Water in the ratio of 90:10 was give good and symmetric peak shape and finalized as mobile phase.

Determination of solubility of dutasteride: It was observed that dutasteride was soluble in water, and freely soluble in methanol, acetonitrile, 0.1 N HCL, 0.1 N NaOH . dutasteride showed highest stability below pH 7.0. So Diluent chosen was ACN:Water (90:10), which showed good solubility and stability also.

Selection of Column Temperature: An inclusion of column temperature (35°C) has minimized day-to-day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

System suitability: Calculations of System Suitability parameters were present in table 1 and Figure no-3.

Application of developed method to pharmaceutical formulation

The proposed validated method was successfully applied to the sample solution was analyzed in optimized chromatographic condition.

Method validation:[8]

Accuracy:- The % Accuracy is within limit (98.0 – 102.0 %) with %RSD less than 2%.So the method is accurate.

Precision:

Method precision (repeatability):The RSD of Assay of six sample preparations was found to be 2.0%

Linearity and range:- The correlation coefficient value should not be less than 0.995 over the working range.

HPLC method development, optimization and validation of Tamsulosin

Selection of detection wavelength: Drugs showed maximum absorbance at 225 nm. So the wavelength selected for the determination of Tamsulosin was 225 nm.

Determination of solubility of Tamsulosin: It was observed that Tamsulosin was soluble in water, and freely soluble in methanol, 0.1 N HCL, 0.1 N NaOH . Tamsulosin showed highest stability below pH 7.0. So Diluent chosen was Buffer : methanol (45:55), which showed good solubility and stability also.

Selection of Column Temperature: An inclusion of column temperature (35°C) has minimized day-to-day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

System suitability:- Calculations of System Suitability parameters were present in table 6 and Figure no-5.

Application of developed method to pharmaceutical formulation: The proposed validated method was successfully applied to the sample solution was analyzed in optimized chromatographic condition.

Method validation of Tamsulosin:-

Accuracy:- Accuracy for individual and mean at each level should be between 98.0% to 102.0% with RSD not more than 2.0%.

Precision:

Method precision (repeatability):The RSD of Assay of five sample preparations should not be more than 2.0%.

Linearity and range:- The correlation coefficient value should not be less than 0.995 over the working range.

CONCLUSION

In this ‘‘Analytical method development and validation of dutasteride with tamsulosin in pharmaceutical capsule dosage form by RP-HPLC method” it include:

- Methods including RP-HPLC, LC-MS, Spectrophotometric etc, were reviewed for the estimation of Dutasteride with Tamsulosin in human plasma as well as pharmaceutical formulations.
- RP-HPLC method for estimation of Dutasteride with Tamsulosin in its dosage form was developed.
- The developed RP-HPLC method was validated for linearity and range, accuracy, method and intermediate precision,system suitability and applied to pharmaceutical formulation.

For the simultaneous estimation of Dutasteride with Tamsulosin is a simple, fast and reliable RP-HPLC method was developed and validated in capsule dosage forms. The developed method was successfully applied for the analysis of Dutasteride and tamsulosin. The method shows a good performance with respect to specificity, linearity, sensitivity, accuracy, precision, selectivity. So the proposed method can be used in routine quality control laboratories.

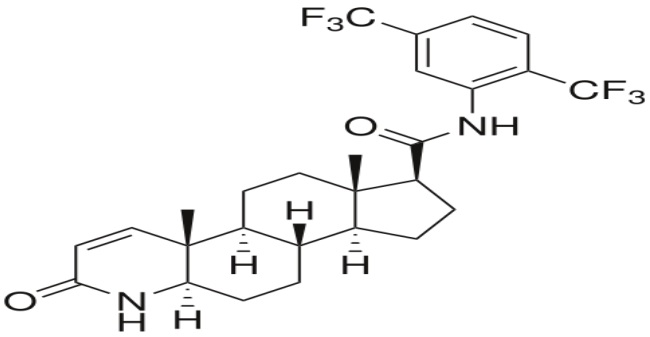


Figure no-1 Structure of Dutasteride

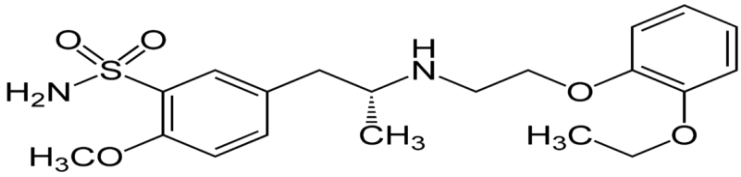
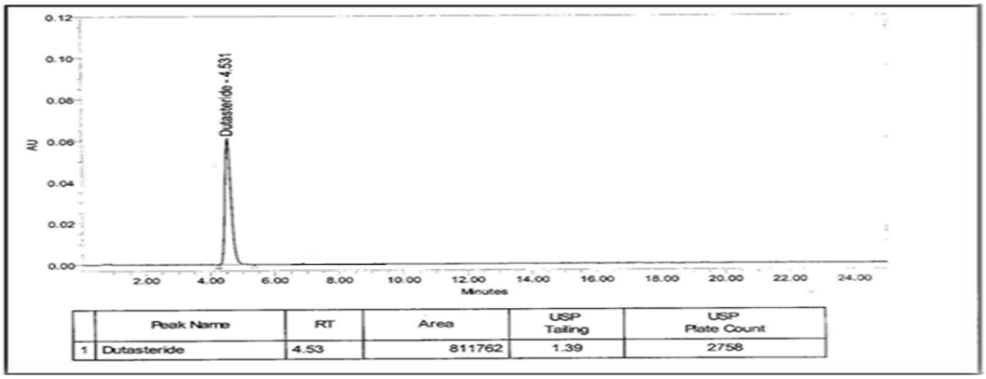


Figure no-2 Structure of Tamsulosin



	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	Dutasteride	4.53	811762	1.39	2758

Figure no-3 Typical Chromatogram of Dutasteride

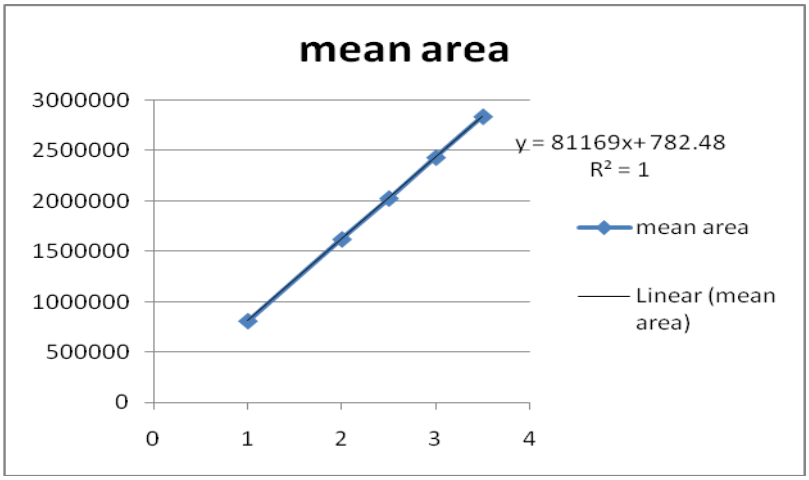
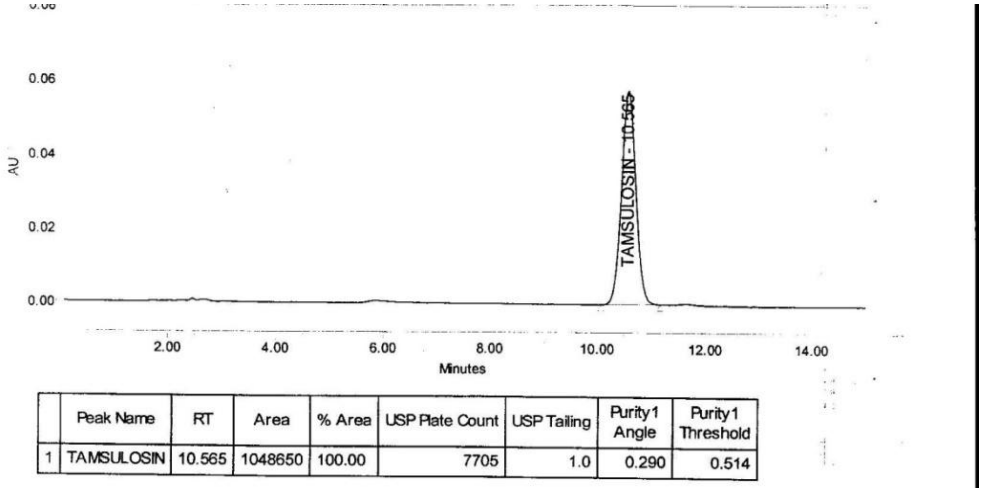


Figure No-4 Calibration curve of dutasteride



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	TAMSULOSIN	10.565	1048650	100.00	7705	1.0	0.290	0.514

Figure no-5 Chromatogram of Standard for System Suitability

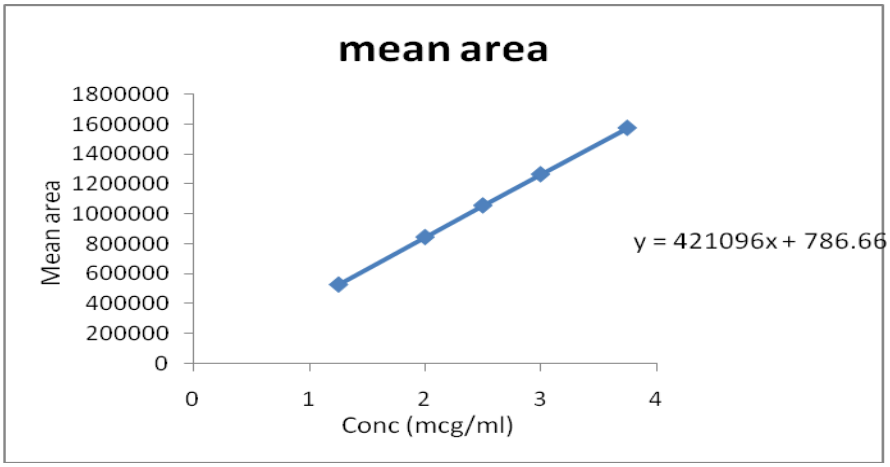


Figure no-6 Calibration curve of Tamsulosin

***Table no-1 System sutability Parameters* of Dutasteride**

S.NO.	RETENTION TIME	AREA	THEORETICAL PLATES	ASSYMETRY
1.	4.571	811699	7772	1.1
2.	4.568	813432	7821	1.3
3.	4.565	819860	6945	1.0
4.	4.559	812498	7124	1.1
5.	4.564	815648	7702	0.9
6.	4.566	816720	7945	1.2

Table no-2 Recovery at 50% level of Dutasteride

Sample No.	Amount spiked (mg)	Amount recovered (mg)	%Recovery
1	1.30	1.32	101.6
2	1.30	1.32	101.6
3	1.30	1.32	101.6

Table no-3 Recovery at 150% level of Dutasteride

Sample No.	Amount spiked (mg)	Amount recovered (mg)	%Recovery
1	3.80	3.83	100.8
2	3.80	3.82	101.1
3	3.80	3.82	101.1

Table no-4 Precision (repeatability) of Dutasteride

Sr. No.	Standard reading	Test reading	Assay (mg/ tab)
1	811763	811699	99.7
2	811693	811734	100.3
3	811760	811767	100.9
4	811766	811766	99.8
5	811683	811757	100.6
6	811753	811772	99.8

Table no-5 Linearity and range of Duteraside

Linearity level	Duteraside	
	Conc.(µg/mL)	Mean area
1	1.00	811699
2	2.00	1623398
3	2.50	2029248
4	3.00	2435097
5	3.50	2840947
Correlation coefficient(R^2)	1.0000	
Slope of regression line	81169	
Y-intercept	782.48	

Table no-6 Calculation of System Suitability of Tamsulosin

S.NO.	RETENTION TIME	AREA	THEORETICAL PLATES	ASSYMETRY
1.	10.571	1046068	7770	1.0
2.	10.568	1045255	7816	1.0
3.	10.565	1048650	7705	1.0
4.	10.559	1045012	7823	1.1
5.	10.564	1047282	7769	1.0
6.	10.566	1046739	7814	1.0

Table no-7 Recovery at 50% level of Tamsulosin

Sample No.	Amount spiked (mg)	Amount recovered (mg)	%Recovery
1	1.25	1.26	101.6
2	1.25	1.26	101.6

Table no-8 Recovery at 150% level of Tamsulosin

Sample No.	Amount spiked (mg)	Amount recovered (mg)	%Recovery
1	3.75	3.76	100.8
2	3.75	3.77	101.1

Table no-9 Precision (repeatability) of Tamsulosin

Sr. No.	Standard reading	Test reading	Assay (mg/ tab)
1	1048022	1054584	99.7
2	1048022	1060690	100.3
3	1048022	1065156	100.9
4	1048022	1056138	99.8
5	1048022	1064570	100.6
6	1048022	1056557	99.8

Table no-10 Linearity and range of Tamsulosin

Linearity level	Tamsulosin	
	Conc. (µg/mL)	Mean area
1	1.25	524893
2	2.00	843648
3	2.50	1056142
4	3.00	1265694
5	3.75	1577252
Correlation coefficient(R^2)	1.0000	
Slope of regression line	421096	
Y-intercept	786	

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