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METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TAMSULOSIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UPLC

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ABSTRACT:
A simple, Précised, Accurate method was developed for the estimation of Tamsulosin by RP-UPLC technique. Chromatographic conditions used are stationary phase Hibra C18 100mm x 1.8 mm, 2.6µ.,Mobile phase0.01N KH_2PO_4 (3.2 pH): Acetonitrile in the ratio of 60:40 and flow rate was maintained at 0.3ml/min, detection wave length was 224nm, column temperature was set to 30°C. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.4 for intermediate precision.LOD and LOQ are 0.039µg/ml and 0.120µg/ml respectively. By using above method assay of marketed formulation was carried out 100.23% was present. Degradation studies of Tamsulosin were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Key words: *UPLC, Tamsulosin, Method development, ICH Guidelines.*

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I. 1.INTRODUCTION

Tamsulosin chemically it is known as 5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-methoxybenzene-1-sulfonamide.[1-5] It 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 [6-8].

Structure:

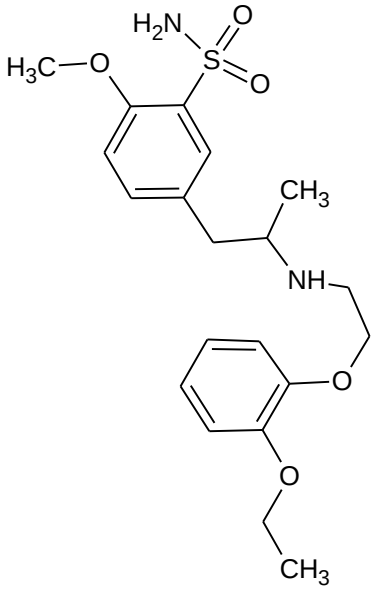

Fig No:1.Tamsulosin structure

Literature review reveals that different methods RP-HPLC [11-17], UV[18-19], LCMS[20-24], for its analysis in formulations. Hence our present plan is to develop a new, sensitive, robust& accurate method for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms [9, 10].

II. MATERIALS AND METHODS

2.1 Apparatus and chromatographic parameters:

UPLC instrument used was of WATERS UPLC SYSTEM with Auto Injector and Acquity TUV detector. Software used is Empower 2. UV-Visible spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Tamsulosin solutions, Sonicator (Ultrasonic sonicator)**,** P^H meter (Thermo scientific)**,** Micro balance (Sartorius)**,**Vacuum filter pump.

2.2. Drug samples and Reagents:

Tamsulosin pure drugs (API) and Tamsulosin tablets [Flomax], Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem Laboratories Ltd.,

2.3. Analytical Methodology:

2.3.1. Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

2.3.2. Preparation of Standard stock solutions: Accurately weighed 4mg of Tamsulosin transferred to50ml volumetric flasks; 25ml of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution. 1ml of Tamsulosin from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (8µg/ml of Tamsulosin).

2.3.3.Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 8ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. 5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (8µg/ml of Tamsulosin)

2.3.4. Preparation of buffer:

Buffer: 0.01N Potassium dihyrogen ortho phosphate:

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.2 with dil. Ortho phosphoric acid solution

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water

2.3.5. Mobile phase: 0.01N KH_2PO_4 (3.2 pH): Acetonitrile (60:40)

2.3.6. Linearity Sample Preparation: Accurately weighed 4mg of Tamsulosin transferred 50ml and volumetric flasks, 25ml of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution (80µg/ml of Tamsulosin).

III. RESULTS AND DISCUSSION:

3.1Method development:

Based on drug solubility and P^{ka} Value following conditions has been used to develop the method estimation of Tamsulosin.

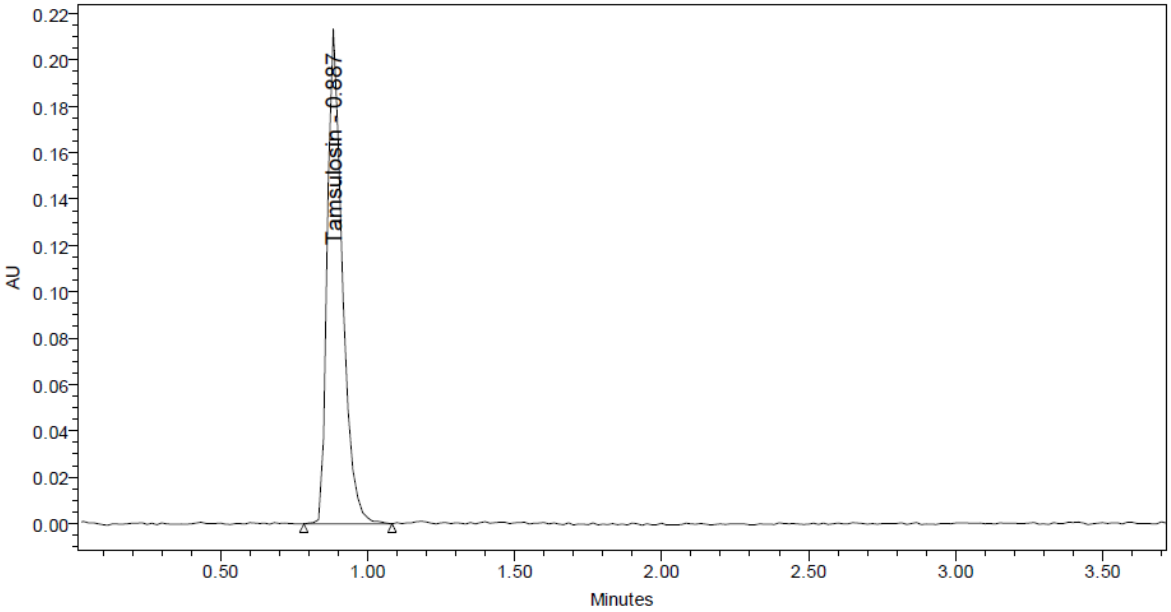

Fig No:2 optimized chromatogram

Table No: 1.Optimized Chromatographic Condition

Parameter	Content
Column	Hibra C18 100mm x 1.8 mm, 2.6µ.
Mobile Phase	0.01N KH_2PO_4 (3.2 pH): Acetonitrile (60:40)
Flow Rate	0.3 ml/min
Temperature	30^0C
Injection Volume	0.50µL
Detection & Wavelength	Acquity TUV 224nm

3.2. Specificity / selectivity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of this drug in this method. So this method was said to be specific.

Table No: 2. Specificity data

SL.No	Peak Name	RT	Area	%Area	USP Plate Count	USP Tailing
1	Tamsulosin	0.884	764803	100.00	2414	1.33
2	Tamsulosin	0.885	758916	100.00	2423	1.33
3	Tamsulosin	0.886	764458	100.00	2370	1.35
4	Tamsulosin	0.890	749850	100.00	2373	1.41
5	Tamsulosin	0.891	758407	100.00	2412	1.42
6	Tamsulosin	0.892	762390	100.00	2425	1.46
Mean			759804			
Std.Dev.			5570.7			
%RSD			0.7			

IV. METHOD VALIDATION:

4.1 Linearity:

To demonstrate the linearity of assay method, injected 6 standard solutions with concentrations of about 2 ppm to 12 ppm of Tamsulosin and Plotted a graph to concentration versus peak area. Slope obtained was 93662, Y-Intercept was 2127 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig 3.

Table No: 3. Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	2	188118
50	4	379493
75	6	560890
100	8	760243
125	10	939283
150	12	1120660

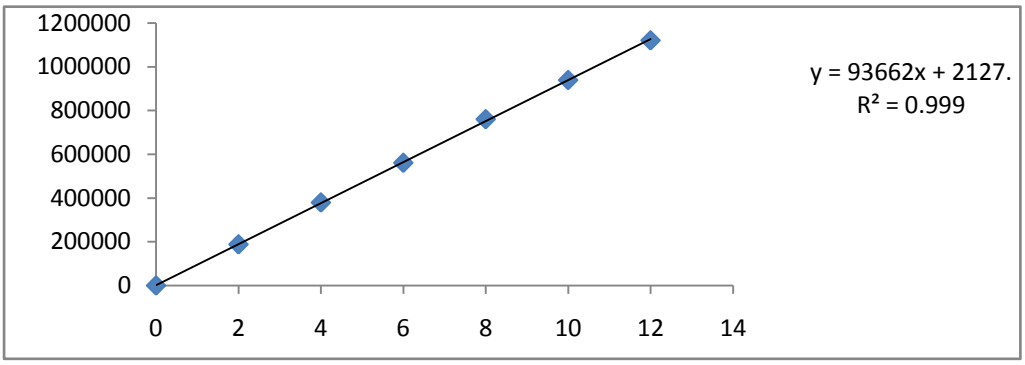

Fig No:3. Linearity Plot

4.2. Precision and Intermediate precision:

Six working sample solutions of 8ppm were injected and the % Amount found was calculated. The %RSD was found to be 0.6 is within the acceptable range.

Six working sample solutions of 8ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.4 is within the acceptable range.

Table No: 4. Precision and Intermediate precision data

S.No	Precision	Intermediate precision
1	764927	751313
2	755224	748239
3	760208	755940
4	768814	751410
5	761268	752419
6	763284	755356
AVG	762288	752446
STDEV	4600.3	2854.2
%RSD	0.6	0.4

4.3. Accuracy:

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.57%.

Table No: 5.Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	4	4.05	101.35	100.57%
	4	4.07	101.64	
	4	3.97	99.17	
100%	8	8.01	100.18	
	8	8.06	100.79	
	8	7.98	99.79	
150%	12	11.95	99.60	

	12	12.09	100.75	
	12	12.22	101.80	

4.4. Robustness:

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions were calculated and tabulated below.

Table No: 6. Robustness Data

PARAMETER	%RSD	TAMSULOSIN
Flow rate (±0.2ml/min)	0.8mL/min	0.6
	1.2mL/min	0.6
Mobile phase(±5)	70:30	0.2
	60:40	0.5
Temperature(±2°C)	28°C	0.6
	32°C	0.6

4.5. Limit of detection and Limit of quantification:

Detection limit of the Tamsulosin in this method was found to be 0.039µg/ml. Quantification limit of the Tamsulosin in this method was found to be 0.120µg/ml.

V. DEGRADATION

5.1. Degradation Studies:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

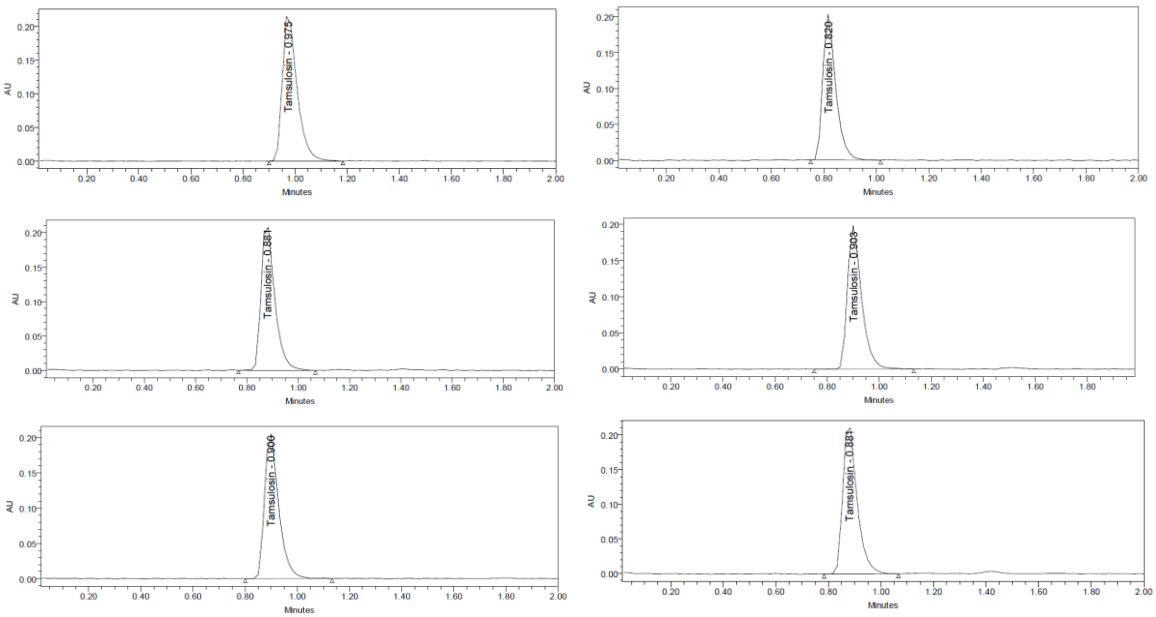

Fig No: 4. Degradation Chromatograms of Tamsulosin

Table No: 7.Degradation Data of Tamsulosin

S.NO	Degradation Condition	% Drug Degraded
1	Acid	6.53
2	Alkali	6.63
3	Oxidation	3.00
4	Thermal	2.27
5	UV	1.38
6	Water	0.95

VI. CONCLUSION

Chromatographic conditions used are stationary phase Hibra C18 100mm x 1.8 mm, 2.6µ,Mobile phase 0.01N KH2PO4: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 0.30ml/min, detection wave length was 224nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between25% to150 % levels, R^2 value was found to be as 0.999.Precision was found to be 0.6 for repeatability and 0.4 for intermediate precision.LOD and LOQ are 0.039µg/ml and 0.120µg/ml respectively. By using above method assay of marketed formulation was carried out 100.23% was present. Degradation studies of Tamsulosin were done, in all conditions purity threshold was more than purity angle and within the acceptable range and this method can be used for routine analysis of Tamsulosin.

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