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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Alfuzosin Hydrochloride and Dutasteride in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid, accurate, specific and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous estimation of Alfuzosin hydrochloride (ALF) and Dutasteride (DUTA) in bulk drug and pharmaceutical dosage form. The chromatographic separation was performed on Thermoscientific Hypersil BDS C18 Column (150mm×4.6mm, 5µm particle size) using a mobile phase of Ammonium dihydrogen phosphate buffer: Acetonitrile (30:70 v/v), at a flow rate of 0.75 ml/min at an ambient temperature with the detection wave length at 292nm. The retention times of ALF and DUTA were 1.27 min and 4.92 min respectively. The linearity was performed in the concentration range of 2.5-15µg/ml (Alfuzosin Hydrochloride) and 0.125-0.75 µg/ml (Dutasteride) with a correlation coefficient of 0.9997 and 0.9998 for ALF and DUTA respectively. The percentage purity of Alfuzosin and Dutasteride was found to be 99.3 and 99.2% w/v respectively. The Proposed method has been validated for specificity, linearity, precision, accuracy and robustness were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the bulk and combined pharmaceutical dosage forms.

Key words: *Alfuzosin Hydrochloride, Dutasteride, RP-HPLC, Validation.*

INTRODUCTION

Alfuzosin Hydrochloride (ALF) is chemically (R,S)-N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride, its molecular weight is 425.91g/mol with an empirical formula $C_{19}H_{27}N_5O_4HCl$. ALF is a selective α_1 adrenoreceptor blocking agent. It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate. Dutasteride (DUTA) is chemically designate as (5α, 17β-N-{2, 5- bis-(trifluoromethyl) phenyl}-3-oxo-4-aza-androst-1-ene-17-carboxamide. DUTA is a synthetic 4-azasteroid compound that is a competitive and selective specific inhibitor of both type 1 and type 2 isoforms of steroid 5-α reductase (5AR), an intracellular enzyme that converts testosterone to 5-α dihydrotestosterone (DHT). The empirical formula of Dutasteride is $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5 g/mol. The combination of these both Alfuzosin Hydrochloride and Dutasteride drugs were used for the treatment of benign prostatic Hyperplasia (BPH).

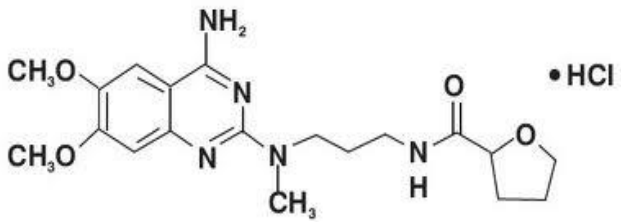

Fig. 1: Chemical structure of Alfuzosin Hydrochloride

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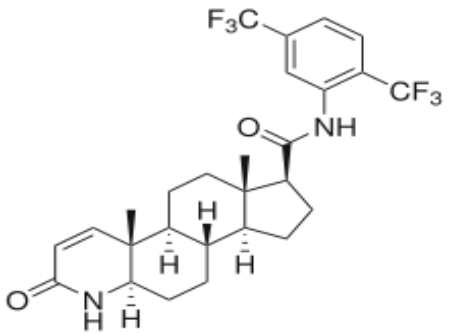

Fig. 2: Chemical structure of Dutasteride

Literature survey reveals that few Spectrophotometric methods [1-2], Colorimetric method [3], HPLC methods [4-8], HPTLC method [9] and has been reported for the estimation of Alfuzosin Hydrochloride and Dutasteride. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Alfuzosin Hydrochloride and Dutasteride in bulk and pharmaceutical dosage form as per ICH guide lines.

MATERIALS AND METHODS

Instrumental and analytical conditions:
Reagents and chemicals:

The pharmaceutical drug samples Alfuzosin Hydrochloride and Dutasteride were obtained as a gift from Cipla Pvt. Ltd. Mumbai (Maharashtra, India), Dr.Reddy's Laboratories, Hyderabad (Andhra pradesh, India), Cadila Pharmaceuticals, Ahmedabad (Gujarat, India)respectively. All the chemicals used of HPLC grade such as Ammonium dihydrogen Phosphate Buffer was obtained from Rankem (RFCL Limited)Manufacturers and Acetonitrile was purchased from Thermo fischer scientific India Pvt. Ltd, used as a mobile phase. Water used in the buffer preparation was freshly prepared from Milli-Q, NA.

Equipment:

A Waters e2695 gradient system with Empower-2 software and 2489 UV/Vis detector is the most sensitive and versatile dual wave length absorbance detector was used. It was manufactured by the company Waters, Alliance, Japan. Intelligent LC pump with sampler programmed at 20µL capacity per injection was used.

Chromatographic conditions:

The column used was Thermoscientific Hypersil BDS C18 Column (150mm×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of an aqueous solution of Ammonium dihydrogen phosphate buffer (pH 4.9) and Acetonitrile in the ratio of (30:70%v/v). The flow was adjusted to 0.75ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 292nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL capacity.

Preparation of Analytical solutions:

Preparation of Ammonium dihydrogen phosphate buffer solution:

A weighed quantity of 0.57515 gm Ammonium dihydrogen phosphate $(NH_3)_4H_2PO_4$ taken in a 1000ml beaker. To this add 500ml of HPLC water and mixed in ultra sonicator and filtered through 0.45µ membrane filter and the resulting solution is having the pH-4.9.

Preparation of Mobile phase:

Mix a mixture of above buffer 300 ml (30%), 700 ml of Acetonitrile (HPLC grade-70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of standard stock solution:

The standard stock solution was prepared by dissolving 10mg of standard drug of Alfuzosin Hydrochloride and 0.5mg of Dutasteride taken in to 100ml volumetric flask to which add 40ml of mobile phase [Ammonium dihydrogen phosphate buffer (pH=4.9): Acetonitrile (30:70 v/v)] and sonicated for about 10 min then the final volume was made upto100 ml with the mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration.

Preparation of standard solution:

Pipette out 10ml from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solution (Marketed formulation):

10 capsules were weighed and the average weight (611.2 mg) was calculated and the sample weight observed is 612.1mg which is having an equivalent to 10mg of Alfuzosin Hydrochloride and 0.5mg of Dutasteride, hence 612.1mg of powder (sample) is taken in to 100ml volumetric flask and add 40ml of mobile phase sonicated for about 10 min and finally make up the volume to 100ml with mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration levels.

Method Development and Validation of HPLC:

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, robustness and system suitability.

Specificity:

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced degradation studies are carried out by using 0.1M HCl, 0.1M NaOH, heat and U.V light.

Linearity:

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of Alfuzosin and Dutasteride, the solutions of six different concentration levels 2.5-15µg/ml (Alfuzosin Hydrochloride) and 0.125-0.75 µg/ml (Dutasteride) are injected in to the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-R^2 and standard error (Er) were determined.

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments).

In order to determine precision, six independent sample solution preparations from a single lot of formulation 10µg/ml for Alfuzosin Hydrochloride and 0.5µg/ml for Dutasteride was injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Accuracy:

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 50%, 100% and 150 % for Alfuzosin and Dutasteride. Standard and sample solutions are injected in to HPLC system in triplicate and percentage recoveries of Alfuzosin and Dutasteride were calculated. The area of each level was used for calculation of % recovery.

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.65ml/min to 0.85ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±5%.

System Suitability:

System suitability tests were carried out on freshly prepared standard stock solutions of Alfuzosin and Dutasteride and it was calculated by injecting standards in six replicates at 6 minutes interval and the values were recorded.

RESULTS AND DISCUSSIONS

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Alfuzosin Hydrochloride and Dutasteride. The method developed was proceeding with wavelength selection. The optimized wavelength was 292nm.

In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consisted of an aqueous solution of Ammonium dihydrogen Phosphate buffer (pH-4.9) and Acetonitrile in the ratio of 30:70% v/v and the column used was Thermo scientific Hypersil BDS C18 Column (150mm×4.6mm,5µm particle size). The flow rate was adjusted to 0.75ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 292nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 2.5-15µg/ml (Alfuzosin Hydrochloride) and 0.125-0.75 µg/ml (Dutasteride). The calibration curve obtained by plotting peak area versus concentration and presented in **Table 1** was linear and the correlation coefficient was found to be 0.9997 and 0.9998 for Alfuzosin Hydrochloride and Dutasteride respectively.

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in **Table 2** was found to be 0.81 and 0.67 and the % Relative Standard Deviation for method precision presented in **Table 3** was found to be 0.84 and 0.65. The % Relative Standard Deviation for ruggedness presented in **Table 4** was found to be 0.66 and 0.64 for Alfuzosin Hydrochloride and Dutasteride respectively.

The accuracy study was performed in 50%, 100% and 150% .The percentage recovery was determined for Alfuzosin Hydrochloride and Dutasteride and was found to be 99.53 and 99.60% presented in **Tables 5 & 6**.

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in **Table 7**. Theoretical plates and tailing factor were observed and were found to be 2947 and 6962 (theoretical plates) and 1.14 and 0.91 (tailing factor) for Alfuzosin Hydrochloride and Dutasteride respectively.

The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table 8**.

Table No. 1: Linearity results for Alfuzosin Hydrochloride and Dutasteride

S. No.	Alfuzosin Hydrochloride		Dutasteride	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	2.5	302571	0.125	27684
2	5	605672	0.25	49989
3	7.5	905238	0.375	72346
4	10	1178920	0.5	94182
5	12.5	1501284	0.625	117440
6	15	1778921	0.75	137951

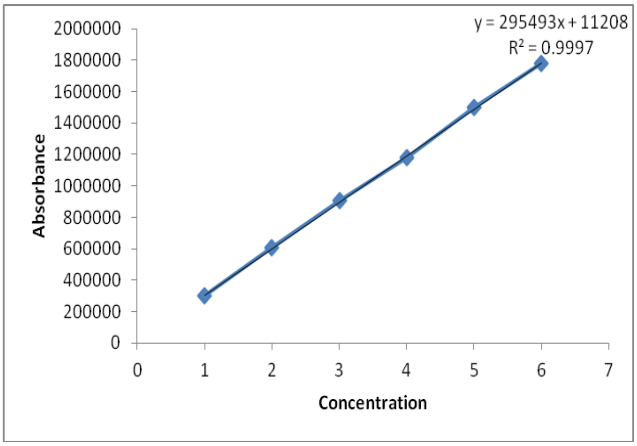

Fig. 3: Linearity plot for Alfuzosin Hydrochloride

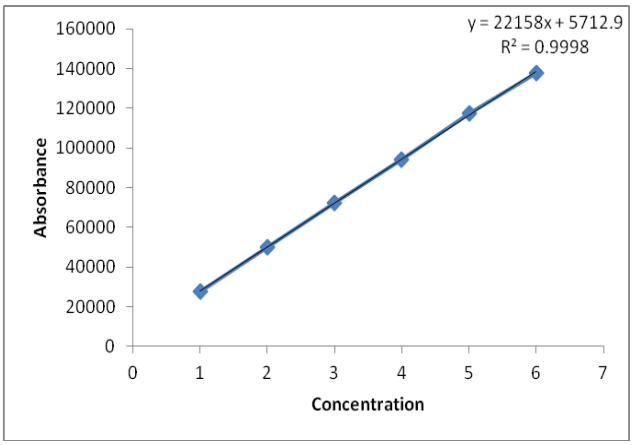

Fig. 4: Linearity plot for Dutasteride

Table No. 2: System Precision values for Alfuzosin Hydrochloride and Dutasteride

Injections	Alfuzosin Hydrochloride		Dutasteride	
	Rt	Area	Rt	Area
1	1.275	1209738	4.946	94639
2	1.277	1188195	4.947	95915
3	1.273	1194990	4.94	95594
4	1.276	1207945	4.939	94196
5	1.277	1187919	4.938	95370
6	1.276	1202964	4.935	95139
Avg.	1.27567	1198625	4.94083	95142.2
Std. Dev.	0.00151	9651.99	0.00471	632.732
%RSD	**0.12**	**0.81**	**0.1**	**0.67**

Table No. 3: Method Precision values for Alfuzosin Hydrochloride and Dutasteride

Injections	Alfuzosin Hydrochloride		Dutasteride	
	R_t	Area	R_t	Area
1	1.272	1198546	4.944	95654
2	1.274	1208542	4.939	95986
3	1.273	1199845	4.938	96846
4	1.275	1180251	4.94	95042
5	1.272	1205463	4.942	96458
6	1.272	1203215	4.935	96024
Avg.	1.2725	1199310	4.94	96001.7
Std. Dev.	0.0017607	10024.5	0.003141	626.692
%RSD	**0.14**	**0.84**	**0.06**	**0.65**

Table No. 4: Ruggedness values for Alfuzosin Hydrochloride and Dutasteride

Injections	Alfuzosin Hydrochloride		Dutasteride	
	Rt	Area	Rt	Area
1	1.275	1186595	4.942	95865
2	1.274	1184632	4.944	96685
3	1.276	1190854	4.945	95532
4	1.277	1199588	4.941	95067
5	1.275	1204587	4.945	96054
6	1.276	1188452	4.948	95147
Avg.	1.2755	1192451	4.944	95725
Std.Dev.	0.00105	7901.79	0.00248	608.891
%RSD	**0.08**	**0.66**	**0.05**	**0.64**

Table No. 5: Recovery Studies for Alfuzosin Hydrochloride

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	591214	5	4.97	99.40%	
100%	1187314.67	10	9.98	99.81%	99.53%
150%	1773452.33	15	14.9	99.39%	

Table No. 6: Recovery Studies for Dutasteride

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	47854.3 95765	0.25	0.24	99.64%	
100%	95765	0.5	0.49	99.69%	99.60%
150%	143343.67	0.75	0.74	99.48%	

Table No. 7: List of Robustness values for Alfuzosin Hydrochloride and Dutasteride

Parameters	Adjusted to	Average area		Rt	
		ALF	DUTA	ALF	DUTA
Flow rate	0.65ml/min	1379528	97661	1.456	5.721
	0.75ml/min	1199321	94877	1.276	4.94
	0.85ml/min	1068312	79219	1.134	4.388
Mobile phase Composition	ACN: Buffer(65:35)	1190744	77114	1.285	6.647
	ACN: Buffer(70:30)	1199321	94877	1.276	4.94
	ACN: Buffer(75:25)	1218047	97553	1.273	3.893

Table No. 8: System suitability Parameters for Alfuzosin Hydrochloride and Dutasteride

S.No.	Parameters	Alfuzosin Hydrochloride	Dutatseride
1	**Average area**	1199321	94877
2	**Retention time(min)**	1.276	4.94
3	**Tailing factor**	1.14	0.91
4	**USP Plate Count**	2947	6962

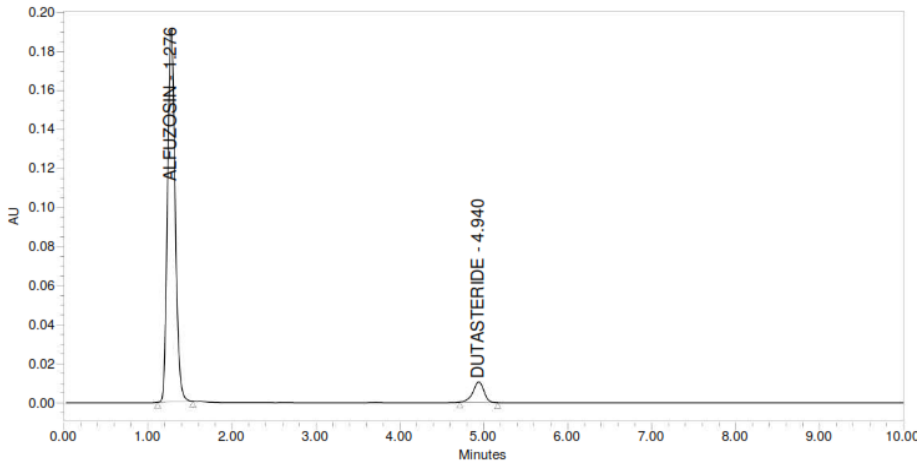

Fig. 5: Standard Chromatogram of Alfuzosin Hydrochloride and Dutasteride

SUMMARY AND CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Alfuzosin Hydrochloride and Dutasteride from API and pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity, and accuracy than previously reported. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Alfuzosin Hydrochloride and Dutasteride in bulk and pharmaceutical dosage form.

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