e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

NEW VALIDATED AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALFUZOSIN AND DUTASTERIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The main purpose of the study was to develop accurate, precise and economic methods for the determination of Dutasteride HCL and Alfuzosin. An HPLC method is developed and validated for various parameters as per ICH guidelines. The system suitability parameters prove that the proposed method is equally suitable for estimation of Dutasteride HCL and Alfuzosin, the chromatogram for Dutasteride and Alfuzosin were found to be satisfactory. The retention time for Dutasteride HCL about 5min, and Alfuzosin was about 2min. The sensitivity of the method is good and also linearity which is observed is good. The accuracy of the method is determined by recovery of drug is well within the acceptance limits of 97-103%. The method is rugged and robust as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio, pH, flow rate, temperature and analysis being performed by different analysts and on different days respectively. In the all above cases the recovery is found to be within the limit.

Keywords: Dutasteride, Alfuzosin, pH, chromatogram, retention time, ruggedness.

INTRODUCTION

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Received:15.11.22 **Revised**:24.11.22 **Accepted**:.28.11.22

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Introduction

Analytical chemistry is a branch of chemistry involved with the analysis of chemical composition of natural and artificial materials. It is the measurement of science consisting of a set of powerful ideas and method that are useful in all fields of science and medicine.

Pharmaceutical analysis is a specialized branch of analytical chemistry which is involved in separating, identifying and determining the relative amounts of components in a sample of matter. Pharmaceutical analysis plays a very important role in quality assurance and quality control of bulk drugs and their formulations. According to WHO, a drug may be defined as any substance or product that is used or intended to be used for modifying or exploring physiological systems or pathological states for the benefit of the patient.

Alfuzosin hydrochloride is termed as (±)-N-[3-[(4-amino-6,7-dimethoxy-2-yl)- methyl-amino propyl] tetrahydro furan -2- carboxamide with the empirical formula C19H27N5O4•HCl and a molecular weight of 389.449g/mol. Alfuzosin 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 [1, 2].

Dutasteride chemically is (5α,17β)-N- {2,5 bis (trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide with an empirical formula C27H30F6N2O2, representing a molecular weight of 528.5 g/mol [3]. It is selective inhibitor of both, type 1 and type 2 isoforms of 5α-reductase (5-AR) enzyme that converts testosterone to 5α-dihydrotestosterone (DHT) which is responsible for enlargement of prostate, is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years [4].

Literature survey revealed RP-HPLC, LC, Selective and rapid liquid chromatography-tandem mass spectrometry, HPTLC and UV spectrophotometric methods for estimation of Alfuzosin hydrochloride and dutasteride in human plasma and pharmaceutical dosage forms 5-16.

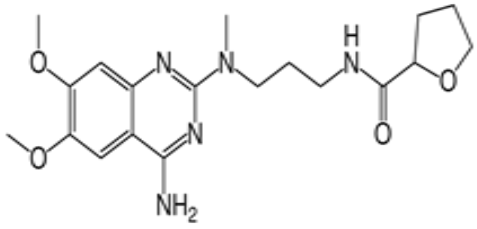

Figure 1: Chemical structure of Alfuzosin Hcl

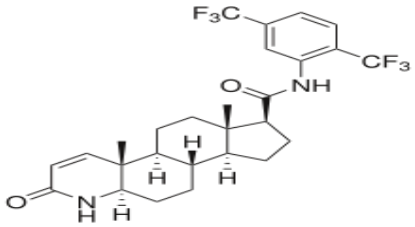

Figure 2: Chemical structure of Dutasteride

MATERIALS & METHODS

Chemicals and Reagents Used:

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Alfuzosin hydrochloride and Dutasteride [Working standards] & KH_2PO_4 all the chemicals were prepared and procured from standard solutions and the tablets were collected from the local market.

Alfuzosin hydrochloride and Dutasteride with the brand name Afudra, contiflo D, Duprost, dutagen, dutalfa, Dutaprost-T, Potassium hydrogen phosphate, methanol, Acetonitrile for HPLC, Ortho phosphoric acid and Distilled Water (HPLC grade).

Apparatus and Chromatographic Conditions:

Table 1: Instruments/equipment used in the study

S. No.	Instruments/equipment	Manufacturer/Supplier
1.	Analytical balance	Citizen
2.	pH meter	Polmon
3.	Ultra sonicator	Bio Technics
4.	HPLC injection syringe	(20µl, stream injector.)
5.	U V	Labindia 3000+
6.	HPLC	Shimadzu Corporation, Japan

Method Development:

The objective of this experiment was to optimize the assay method for simultaneous estimation of Alfuzosin and Dutasteride based on the literature survey made and the methods given in official pharmacopoeias. So here the trials mentioned describes how the optimization was done.

Trails

Table 2: Chromatographic conditions for trail 1 and trail 2

Parameters		Trail 1	Trail 2
Mobile phase	:	Acetonitrile: Water (50:50)	Methanol: Water (80:20)
F low rate	:	1ml/min	**2** ml/min
Column	:	OEM Column (250x4.6mm, 5µ)	Inertsil ODS C18(250x4.6mm, 5µ)
Detector wave length	:	225nm	225nm
Column temperature	:	Ambient	Ambient

Injection volume	:	20 µl	20 µl
Run time	:	35min	30min
Diluent	:	Mobile phase	Mobile phase

Table 3: Chromatographic conditions for trail 3 and trail 4

Parameters		**Trail 3**	**Trail 4**
Mobile phase	:	Acetonitrile: Water (50:50)	0.05M Ammonium Acetate buffer: Methanol. (30:70)
F low rate	:	1.2ml/min	1.5 ml/min
Column	:	SUNFIRE COLUMN C8 (150*4.6* 5µ)	Symmetry C8 (150*4.6* 3.5µ)
Detector wave length	:	225nm	225nm
Column temperature	:	Ambient	Ambient
Injection volume	:	20 µl	20 µl
Run time	:	12min	12min
Diluent	:	Mobile phase (0.01m sodium di hydrogen ortho phosphate buffer: ACN (80:20)	Mobile phase 0.05M Ammonium Acetate buffer: Methanol. (30:70)

Observations:

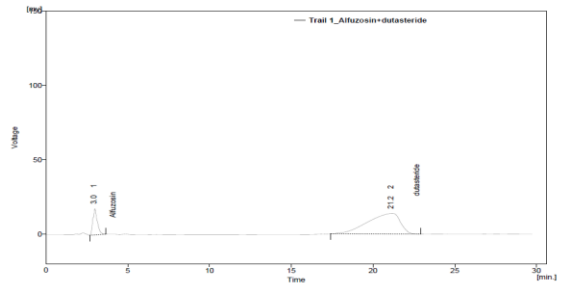

Figure 1: Chromatogram for trail - I

Conclusion: Peaks were not symmetry and eluting times of components were also more.

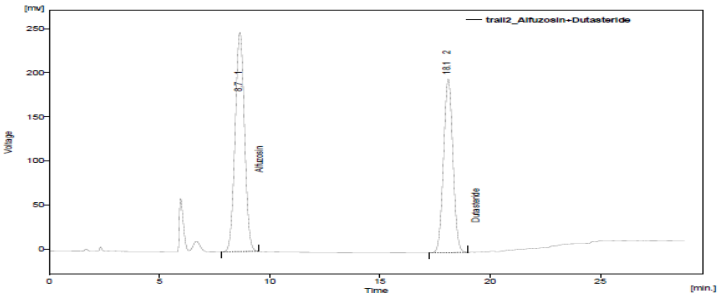

Figure 2: Chromatogram for trail - II

Conclusion: Two Peaks were observed but the retention time of dutasteride is not good.

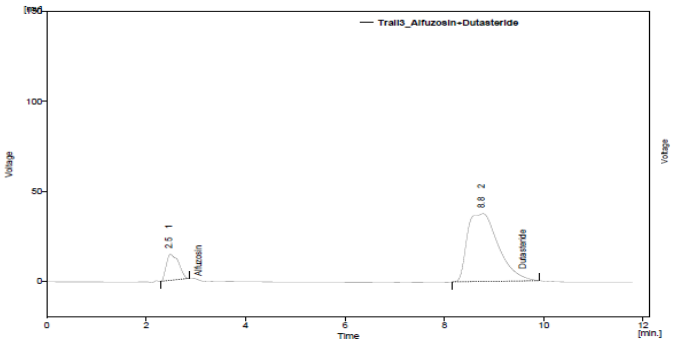

Figure 3: Chromatogram for trail - III

Conclusion: Peaks were observed but efficiency is not good.

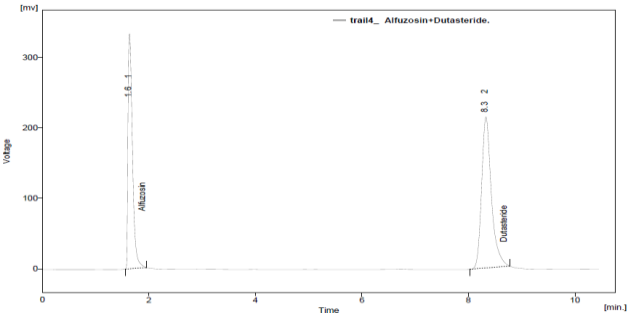

Figure 4: Chromatogram for trail - IV

Conclusion: Peaks were not symmetry.

S.NO	***Name of the peak***	***Retention time(min)***			
		Trail 1	**Trail 2**	**Trail 3**	**Trail 4**
1.	*Alfuzosin*	*2.993*	8.650	2.487	1.633

2.	*Dutasteride*	*21.157*	18.100	8.777	8.327

OPTIMIZED METHOD:

Chromatographic conditions

Preparation of Buffer: Take 1.034gm of Potassium di hydrogen phosphate (0.02M), 0.190 gm of di Potassium Hydrogen ortho phosphate (0.003M). Dissolve in 350 ml HPLC water and sonicated for 10 min. filtered in 0.25 microns in membrane filter.

mobile phase: Filtered and degassed mixture of Acetonitrile and buffer in the ratio of 65:35 and filter through 0.25-micron membrane filter.

Chromatographic conditions

Table 4: Chromatographic conditions for optimized system (Trail 5)

Parameters	**Trail 5**
Mobile phase	Acetonitrile: Water (50:50)
F low rate	1ml/min
Column	C_{18}, 150x4.6mm,5µ universil
Detector wave length	225nm
Column temperature	Ambient
Injection volume	20 µl
Run time	8min
Diluent	Methanol

Observation:

S.NO	**Name of the peak**	**Retention time(min)**
1.	Alfuzosin	2.837
2.	Dutasteride	4.007

The two peaks were well resolved with good peak shape and symmetry. Theoretical plates and asymmetries are within limits.

Conclusion:

Hence this method was finalized for the simultaneous estimation of Alfuzosin and Dutasteride.

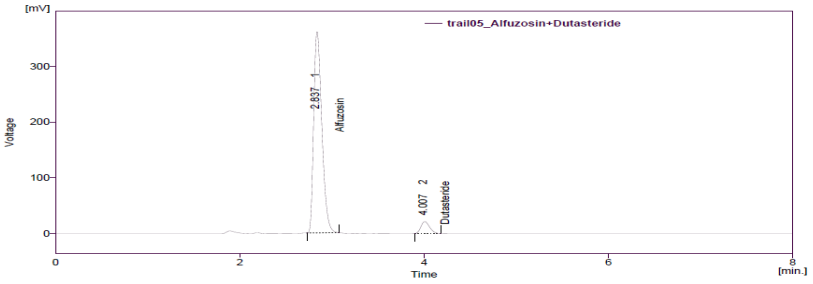

Figure 5: Chromatogram for optimized trail - V

Validation parameter:

System suitability:

According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Various system suitability parameters like plate number (N), asymmetry factor, retention time, resolution, tailing factor, were evaluated from the standard chromatogram.

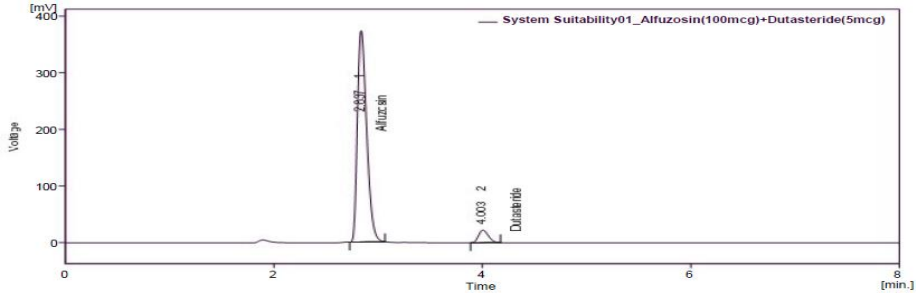

Figure 6: System suitability parameters for Alfuzosin and Dutasteride

Table 5: System suitability parameters for Alfuzosin and Dutasteride

System suitability parameter	Alfuzosin			Dutasteride		
	Retention time	Theoretical plates	Tailing Factor	Retention time	Theoretical plates	Tailing Factor
Solution 1	2.837	4458	1.700	4.03	7804	1.417
Solution 2	2.837	4458	1.619	4.003	7801	1.360
Solution 3	2.837	4458	1.700	4.003	8315	1.360
Solution 4	2.853	4510	1.619	4.043	7960	1.400
Solution 5	2.847	4489	1.750	4.033	7921	1.400
Mean	2.8422			4.017		
S.D	0.00743			0.0194		
R.S.D	0.65768			0.3466		

Method precision :(Repeatability):

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility.

Table 6: Precision of Alfuzosin and Dutasteride

S.No	Alfuzosin		Dutasteride	
	Retention Time(min)	Area	Retention Time(min)	Area
1	2.843	2276.986	4.03	147.127
2	2.85	2271.488	4.04	146.033
3	2.847	2284.55	4.037	147.599
4	2.843	2268.708	4.033	146.778
5	2.843	2299.182	4.03	146.534
Average	2.8452	2280.183	4.034	146.8142
S. D	0.003194	12.22224	0.004416	0.592421
%R. S. D	0.11225	0.53602	0.109467	0.403518

System precision

Table 7: System precision of Alfuzosin and Dutasteride

S.No	Alfuzosin		Dutasteride	
	Retention Time(min)	Area	Retention Time(min)	Area
1	5.888	1823429	5.888	1823429
2	5.886	1819635	5.886	1819635

3	5.892	1826653	5.892	1826653
4	5.893	1819460	5.893	1819460
5	5.896	1812568	5.896	1812568
6	5.897	1827584	5.897	1827584
Average	5.892	1821555	5.892	1821555
S.D	0.004	5562.23	0.004	5562.23
%R.S.D	0.07	0.31	0.07	0.31

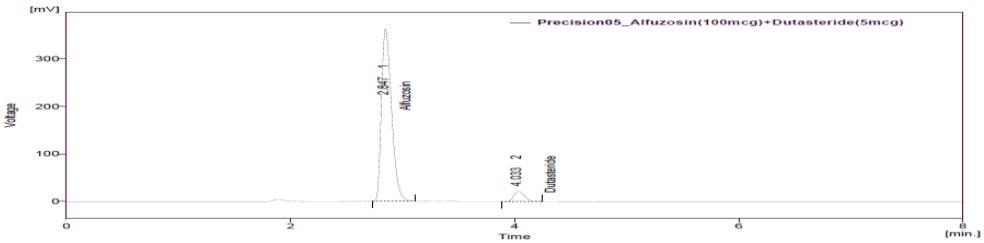

Figure 7: System precision of Alfuzosin and Dutasteride

Precision graph of Alfuzosin and Dutasteride.

Accuracy:

Accuracy is the measure of how close the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

Table 8: Accuracy of Alfuzosin

S.No.	Mixture of pure and formulation	Conc. of pure drug (µg/ml)	Conc.of formulation (µg/ml)	%Recovery of pure drug
1.	80+10	10	89.19	99.1
2.	100+10	10	109.68	99.71
3.	120+10	10	129.69	99.76

Table 9: Accuracy of Alfuzosin

S.No.	Mixture of pure and formulation	Conc. of pure drug (µg/ml)	Conc.of formulation (µg/ml)	%Recovery of pure drug
1.	4+0.5	0.5	4.498	99.95
2.	5+0.5	0.5	5.468	99.41
3.	6+0.5	0.5	6.473	99.59

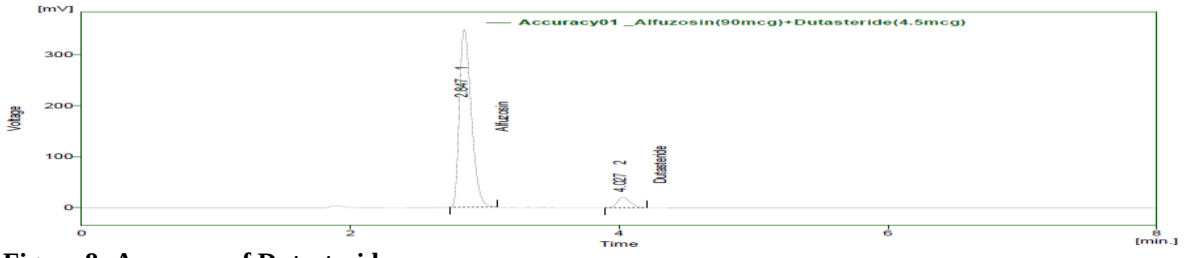

Figure 8: Accuracy of Dutasteride

Linearity

Accuracy is the measure of how close the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

Table 10: Linearity data of Alfuzosin and Dutasteride

S.No	Linearity Level	Concentration (µg/ml)	Volume of stock solution (ml)	Volume made upto (ml)	Peak Area	Concentration (µg/ml)	Volume of stock solution (ml)	Volume made upto (ml)	Peak Area
1	Linearity-1	20	2	100	560.978	1	2	100	34.805
2	Linearity -2	40	4	100	911.535	2	4	100	57.401
3	Linearity-3	60	6	100	1370.185	3	6	100	83.692
4	Linearity-4	80	8	100	1742.885	4	8	100	110.566
5	Linearity-5	100	10	100	2224.455	5	10	100	140.301
6	Linearity-6	120	12	100	2581.977	6	12	100	161.984

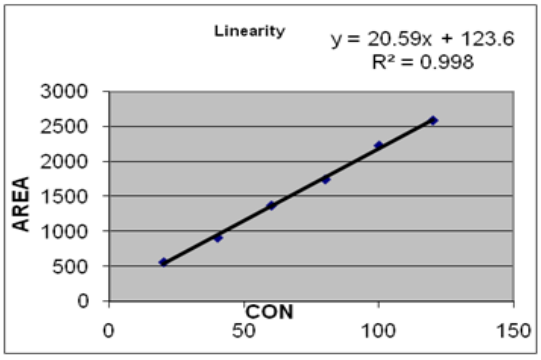

Figure 9: Linearity data of Alfuzosin

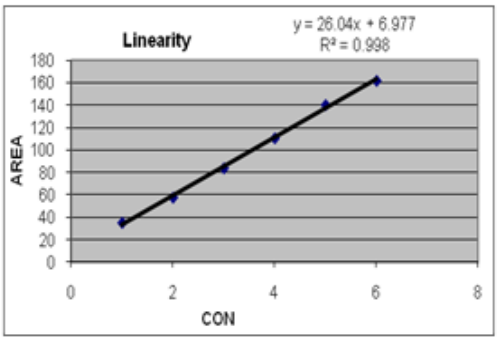

Figure 10: Linearity data of Dutasteride

Acceptance Criteria: r^2 should not be less than 0.99.
Result: The relationship between the concentration and the peak response of Alfuzosin was linear in specific range and the regression coefficient was found to be 0.998. The relationship between the concentration and the peak response of Dutasteride was linear in specific range and the regression coefficient was found to be 0.998.

Specificity

Ability of the method to measure accurately and specifically the analyte of interest in presence of matrix and other components likely to be present in the sample matrix and impurities, degradation products and other related substances.

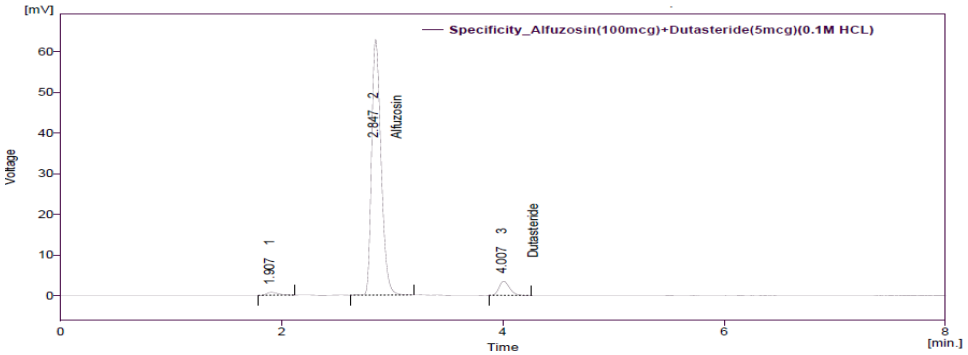

Figure: 11. Specificity-alfuzosin (100 µg) and dutasteride (5 µg) +(0.1m Hcl)

Robustness

It is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Effect of variation of Flow rate
Effect of variation of wavelength

S. No	Chromatographic condition	Low	High
1.	Flow rate	0.9 ml/min	1.1 ml/min
2.	Wavelength	223 nm	227 nm

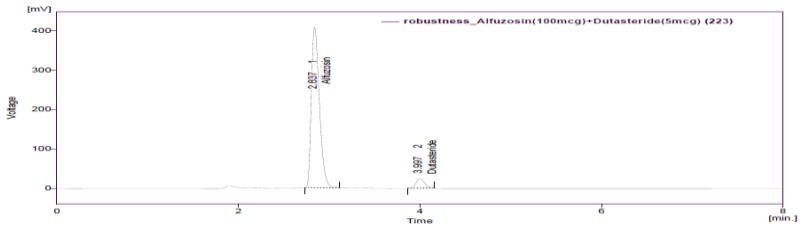

Figure 12: Effect of variation of wavelength

Ruggedness

Intermediate Precision or Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments.

Standard-A:

Accurately weighed quantity of 50 mg Alfuzosin was transferred in to a 100 ml volumetric flask. Dissolved in methanol and sonicated about 10min until all the contents has dissolved, then the volume was made up to the mark with mobile phase.

Standard-B:

Accurately weighed quantity of 2.5mg Dutasteride was transferred in to a 100 ml volumetric flask. Dissolved in methanol and sonicated about 10min until all the contents has dissolved, then the volume was made up to the mark with mobile phase.

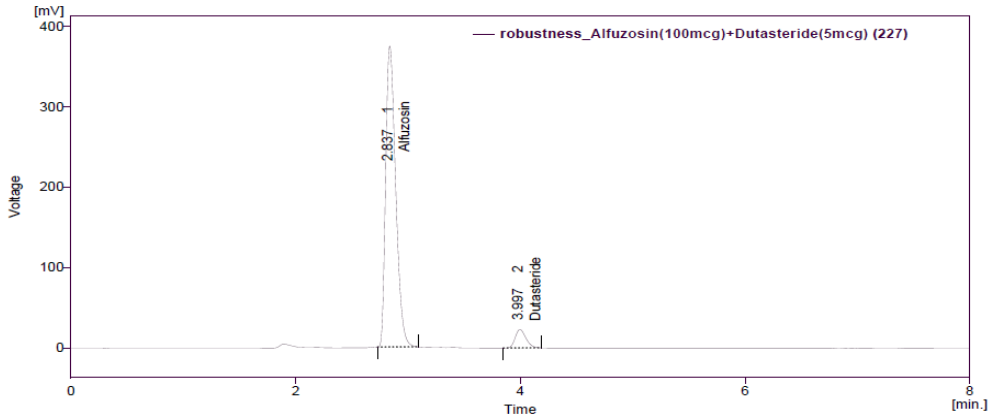

Figure 13: Ruggedness-alfuzosin and dutasteride

Preparation of test solution:

About 202.3mg of the sample was taken in 25ml volumetric flask and added 10ml methanol and sonicated about 10 min and make up with mobile phase. From this 5ml was pipetted out into 20ml volumetric flask and made up to the volume with same mobile phase. Filter the content by using 0.25µ membrane filter by applying vacuum the results are tabulated in Table No.-8 (Fig-37-38).

Procedure:

The standard solution (100mcg) was injected for by different analysts and the area for injections in HPLC was measured. The %RSD for the area of replicate injections was found to be within the specified limits.

Table 12: Ruggedness of Alfuzosin and Dutasteride

	Alfuzosin				Dutasteride			
	Retention time		Area		Retention time		Area	
Inj	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2

1	2.843	2.837	2276.986	2276.97	4.03	4.02	147.127	147.126
2	2.85	2.78	2271.488	2270.79	4.04	4.03	146.033	146.022
3	2.847	2.846	2284.55	2283.98	4.037	4.026	147.599	147.589
Mean	2.846667	2.821	2277.675	2277.247	4.034	4.025333	146.9197	146.9123
S.D	0.003512	0.035791	6.558175	6.599351	0.004416	0.005033	0.803324	0.805054
% R.S.D	0.123368	1.268737	0.287933	0.289795	0.109467	0.125039	0.546778	0.547983

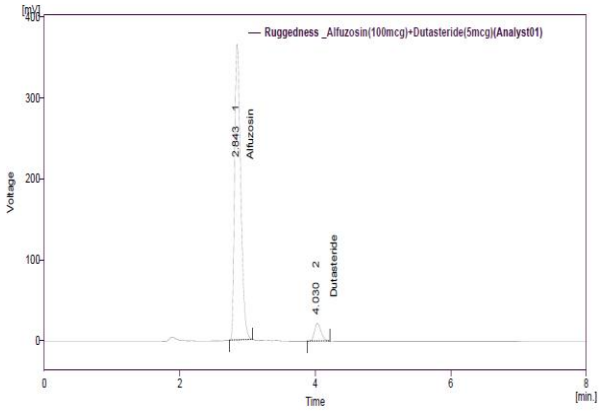

Figure 14: Retention time and Area of Alfuzosin

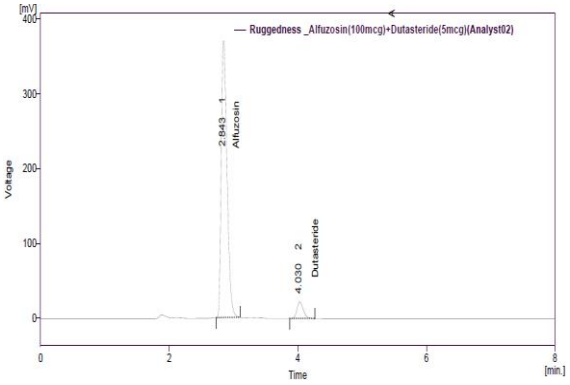

Figure 15: Retention time and Area of Dutasteride

Conclusion

- An RP-HPLC method was developed and validated successfully for simultaneous estimation of Alfuzosin and Dutasteride in Pharmaceutical dosage form.
- The method was found to be accurate, precise, linear, specific and reproducible for the simultaneous determination of Alfuzosin and Dutasteride in formulation.
- Hence this study can be extended by studying the degradation kinetics of Alfuzosin and Dutasteride determination by RP-HPLC method and also its estimation in plasma and biological fluids.

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Cite this article:
P. Lavanya*, B.V. Narasimha Rao, B. Appa Rao**.** T NEW VALIDATED AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALFUZOSIN AND DUTASTERIDE IN PHARMACEUTICAL DOSAGE FORM. *International Journal of Pharmaceutical Research & Analysis*, 2017;7(1):65-74