RESEARCH PAPER

A NOVEL STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ALFUZOSIN HYDROCHLORIDE AND TADALAFIL

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ABSTRACT

A simple, rapid, accurate, precise, and stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Alfuzosin and Tadalafil in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved on a C18 column using a mobile phase of buffer and acetonitrile (65:35, v/v) at a flow rate of 0.6 mL/min under optimized conditions. The method was validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The method showed excellent linearity over the concentration range of 2–240 µg/mL for Alfuzosin and 1–120 µg/mL for Tadalafil, with correlation coefficients of 0.9992 and 0.9997, respectively. Accuracy was found in the range of 100.14–101.82% for Alfuzosin and 99.87–101.37% for Tadalafil, while %RSD for precision was below 2%. The LOD and LOQ values were 0.03 and 0.05 µg/mL for Alfuzosin and 0.05 and 0.07 µg/mL for Tadalafil, respectively. Forced degradation studies confirmed the stability-indicating nature of the method. Therefore, the proposed method is suitable for routine quality control analysis of combined tablet dosage forms.

Keywords: Stability indicating method, Novel RP-HPLC method, Alfuzosin Hydrochloride, Tadalafil, Validation

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INTRODUCTION

The prevalence of lower urinary tract symptoms (LUTS) increases with age and includes both storage and voiding symptoms [1]. Storage symptoms are often associated with detrusor overactivity, while voiding symptoms may result from reduced detrusor contractility or bladder outlet obstruction due to benign prostatic hyperplasia (BPH) [2]. Among the available treatments, α1-adrenergic antagonists are considered effective monotherapy for managing LUTS, with Alfuzosin offering advantages such as good cardiovascular tolerance and minimal impact on sexual function [3].

Phosphodiesterase type 5 inhibitors, including Tadalafil, are widely used for erectile dysfunction (ED) and have also shown benefits in improving LUTS [4–6]. Epidemiological studies indicate a strong association between LUTS and ED, supporting the use of combination therapy [7]. The combined use of alfuzosin and tadalafil has demonstrated improved therapeutic outcomes compared to monotherapy, with no significant hemodynamic interaction observed [8]. Additionally, studies have reported additive relaxant effects of this combination on smooth muscle tissues of the corpus cavernosum, prostate, and bladder, further supporting its clinical utility [9,10].

Alfuzosin and Tadalafil is found in tablet dosage forms. Various analytical methods are described for the estimation of the combination. A simple UV spectrophotometric and spectrofluorometric has been developed for alfuzosin and tadalafil in bulk, tablet dosage form, spiked biological samples [11-14]. One solvent-free mixed-micellar liquid chromatography method was developed to simultaneously analyze alpha-1 blockers alfuzosin (ALF) and tamsulosin (TMS) and phosphodiesterase-5 inhibitor tadalafil (TAD) [15]. One HPLC and TLC method has been described for the estimation of alfuzosin and tadalafil in bulk and pharmaceutical dosage forms [16]. Although a stability indicating HPLC method for the simultaneous estimation of alfuzosin and tadalafil has been previously reported, certain limitations are evident in the published work [17]. The reported method does not include detailed stability-indicating studies supported stability graphs. In the present study, a stabilityindicating RP-HPLC method has been developed and validated that that demonstrates better LOD, LOQ and range. Furthermore, the previously reported method presents identical calibration curves for both drugs, which is analytically questionable since different compounds typically exhibit different detector responses. In contrast, the proposed method establishes

independent calibration curves with wider and more appropriate linearity ranges of 1–120 ppm for tadalafil and 2–240 ppm for alfuzosin.

Stability studies are conducted to evaluate changes in drug substances over time under various environmental conditions such as temperature, humidity, and light [18]. Factors like manufacturing conditions, batch variations, and formulation components can influence drug stability. The development of stability-indicating methods is essential to detect degradation products and ensure drug quality, often achieved through forced degradation studies due to limited availability of naturally degraded samples. These studies play a crucial role in understanding degradation pathways and are important for establishing appropriate storage conditions, shelf life, and expiry as per Good Manufacturing Practice (GMP) guidelines [19].

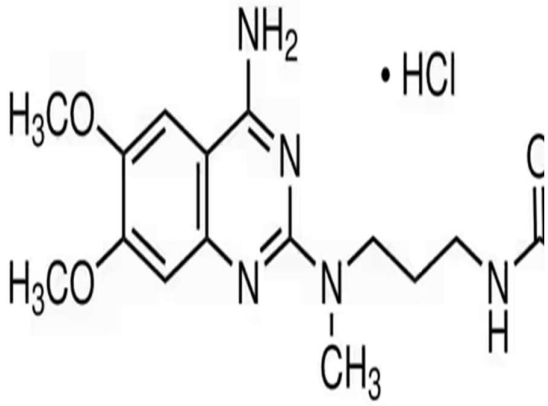

Figure No. 1: Chemical Structure of Alfuzosin HCl (ALFU)

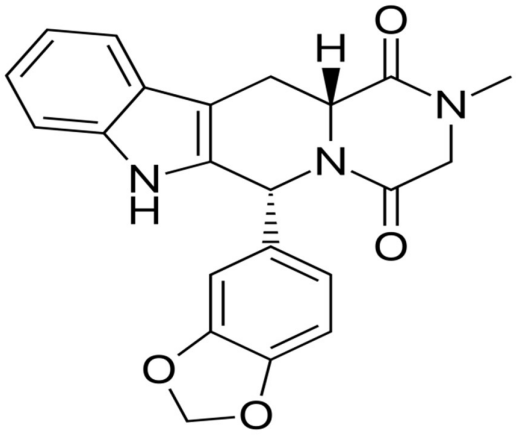

Figure No. 2: Chemical Structure of Tadalafil (TADA)

MATERIALS AND METHODS

Reagents and chemicals: ALFU and TADA were generously supplied as a complimentary sample by Hetero Drug Ltd. (Unit-I), Sangareddy, Telangana State, India. HPLC-grade methanol was purchased from Thermo Fisher Scientific India Pvt. Ltd. High purity water was obtained using an in-house Milli-Q system for chromatographic analysis The marketed formulation Alfoo T by Dr. Reddy's was purchased from local market of Gujarat. All weighing was carried out using NABL certified balances. Sample preparation was carried out using an analytical balance and class A glassware for accuracy and precision.

Instruments used: The analytical instruments employed during the study included a Shimadzu HPLC system (AUX 220), a UV–Visible double-beam spectrophotometer (UV-1800, Shimadzu), an analytical balance (MAB 220, Wensar), an FTIR spectrophotometer (ALPHAE), an ultra-sonicator (CB2080), and a pH meter (pH Tutor, Eutech Instruments Cyber Scan).

Preparation of standard solution: The standard stock solution was prepared by dissolving 100 mg of Alfuzosin and 50 mg of Tadalafil in acetonitrile and diluting to 100 mL to obtain 1000 ppm and 500 ppm, respectively. A working solution was prepared by diluting 5 mL of this stock to 50 mL with acetonitrile to obtain 100 ppm of Alfuzosin and 50 ppm of Tadalafil.

Preparation of sample solution: Tablet powder equivalent to 100 mg of Alfuzosin and 50 mg of Tadalafil was extracted with acetonitrile, sonicated, and diluted to 100 mL to obtain the stock solution (1000 ppm and 500 ppm). Further dilution of 5 mL to 50 mL with acetonitrile yielded the sample solution containing 100 ppm of Alfuzosin and 50 ppm of Tadalafil.

Preparation of buffer: A buffer solution was prepared by dissolving 1.5601 g of sodium dihydrogen orthophosphate dihydrate in 1000 mL of water, and the pH was adjusted to 3.5 using orthophosphoric acid (OPA).

Preparation of mobile phase: The mobile phase was prepared by mixing buffer and HPLCgrade acetonitrile in the ratio of 65:35 (v/v).

Selection of diluent: Acetonitrile was selected as diluent based on solubility and mobile phase composition.

Method development: Several chromatographic experiments were carried out using various columns and mobile phase compositions. Multiple trials were conducted to optimize the chromatographic conditions by altering solvent ratios, flow rate, and column temperature. These parameters were carefully evaluated to obtain suitable retention time (RT), improved peak shape, a higher number of theoretical plates, and an acceptable tailing factor for the analytes. The final optimized chromatographic conditions are illustrated in Fig. 3. Chromatographic separation was achieved using a Waters Xterra C18 150 mm 4.6 mm x 3.5µ. The mobile phase consisted of ACN: Buffer (pH 3.5) (40:60). The prepared mobile phase was pumped at a flow rate of 0.8 mL/min. The column temperature was maintained at 40°C. Detection was performed using a PDA detector at 215 nm. A sample volume of 10 µL was

injected, and the chromatographic run was completed within 20 minutes. System suitability was evaluated by injecting six replicate standard preparations, and the %RSD of the peak area, retention time, height, theoretical plate, tailing factor and resolution response for Alfluzosin and Tadalafil was found to comply with the acceptance criterion of not more than 6.0%.

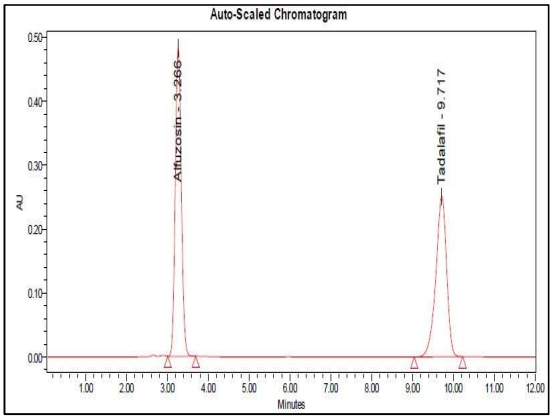

Figure No. 3: Optimized chromatogram of alfuzosin and tadalafil

Forced degradation studies: It was performed in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines to evaluate the stability of Alfuzosin and Tadalafil under various stress conditions.

Acid and alkali degradation were carried out by treating 1 mL of standard stock solution with 2 N HCl (5 h) and 0.5 N NaOH (3 h), respectively, followed by neutralization and dilution to 10 mL with acetonitrile. Oxidative degradation was performed using 30% hydrogen peroxide for 3 hours and diluted similarly. Thermal degradation was conducted by exposing the drug mixture (100 mg Alfuzosin and 50 mg Tadalafil) to 120°C for 24 hours, followed by dilution. Photolytic degradation involved exposure of the standard stock solution to light for 24 hours before dilution.

Validation of the developed method: The developed RP-HPLC method was validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for parameters such as specificity, linearity, accuracy, precision, robustness, LOD, and LOQ.

Linearity: Linearity was evaluated by preparing a series of standard solutions at different concentration levels by diluting the stock solution with methanol.

Repeatability: Repeatability was assessed by six replicate injections of the standard solution (100 ppm Alfuzosin and 50 ppm Tadalafil), and %RSD of peak areas was calculated.

Intraday Interday Precision: Precision was determined at 80%, 100%, and 120% levels by analyzing samples within the same day and on different days, and %RSD was calculated.

Robustness: Robustness was studied by introducing small deliberate changes in chromatographic conditions such as mobile phase, flow rate, and temperature, and observing their effect.

Accuracy: Accuracy was evaluated at 80%, 100%, and 120% levels by preparing solutions from the standard stock and determining the percentage recovery.

RESULTS AND DISCUSSION

Forced degradation studies: The forced degradation study revealed that both drugs were more susceptible to acidic and oxidative stress conditions. In acidic degradation, Alfuzosin and Tadalafil showed 13.26% and 22.13% degradation, respectively. Under oxidative conditions, the degradation increased to 18.20% for Alfuzosin and 26.98% for Tadalafil. In alkaline conditions, lower degradation was observed, with 7.76% for Alfuzosin and 17.61% for Tadalafil. Thermal degradation caused 9.88% degradation of Alfuzosin and 7.24% degradation of Tadalafil, while negligible degradation was observed under photolytic conditions. The purity angle for all stressed samples was found to be lower than the purity threshold, confirming peak purity and indicating that the developed method is stability-indicating.

Table No. 1: Overall result of forced degradation studies

Condition	Drug	Alfuzosin	Tadalafil	% Degradation of Alfuzosin	% Degradation of Tadalafil	Purity Angle	Purity Threshold
Acidic	Alfuzosin	4736672(STD Area)	4397360	86.74	77.87	0.166	0.304
	Tadalafil	4108455(Observed)	3424153	13.26	22.13	0.151	0.216
Oxidative	Alfuzosin	4736672	4397360	81.8	73.02	0.129	0.279
	Tadalafil	3874421	3210939	18.2	26.98	0.034	0.212
Alkali	Alfuzosin	4736672	4397360	92.24	82.39	0.117	0.391
	Tadalafil	4369078	3623200	7.761	17.61	0.048	0.233

Thermal	Alfuzosin	4736672	4397360	90.12	92.75	0.105	0.353
	Tadalafil	4268513	4078650	9.884	7.248	0.036	0.220
Light or UV	Alfuzosin	4736672	4397360	100.5	100.8	0.095	0.290
	Tadalafil	4760016	4434253	-0.49	-0.84	0.170	0.218

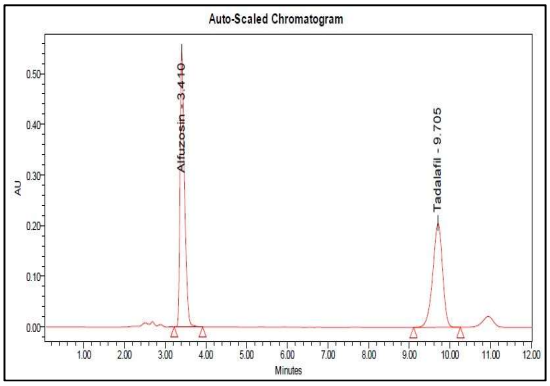

Figure No. 4: Chromatogram

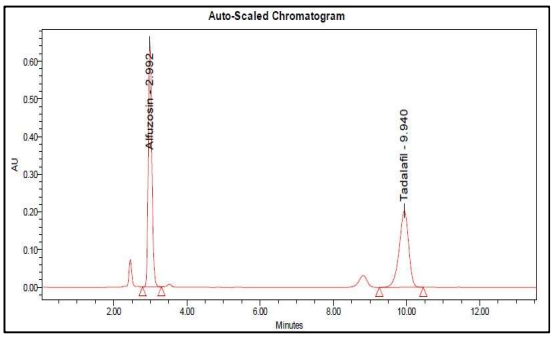

Figure No. 5: Chromatogram for Alkali hydrolysis

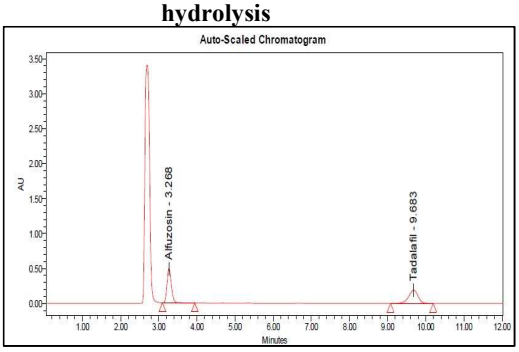

Figure No. 6: Chromatogram for Oxidative stress

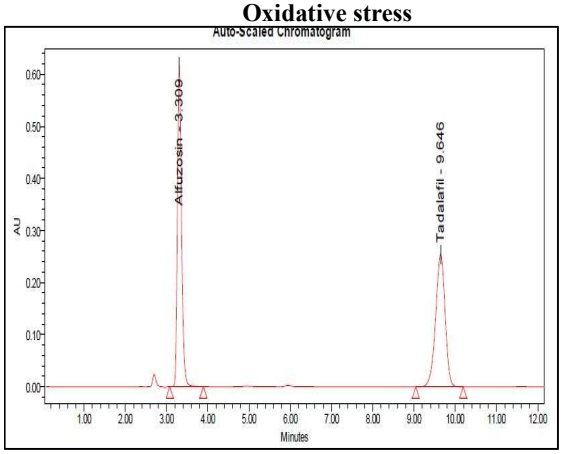

Figure No. 7: Chromatogram for Thermal stress

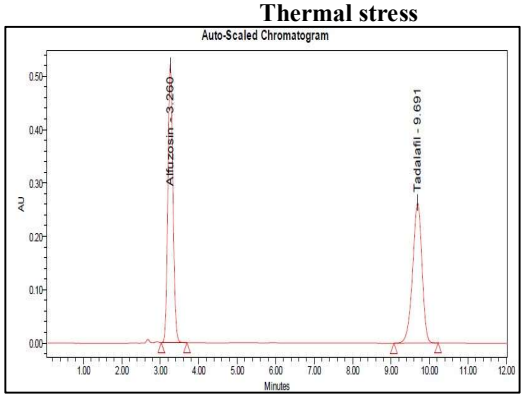

Figure No. 8: Chromatogram for Light degradation Validation of the developed method: The linearity of the developed RP-HPLC method was evaluated by analyzing standard solutions over the concentration range of 2–240 ppm for Alfuzosin and 1–120 ppm for Tadalafil. Alfuzosin exhibited linearity at concentrations of 2, 10, 20, 80, 160, and 240 ppm with a correlation coefficient (R^2) of 0.9992, while Tadalafil showed linearity at concentrations of 1, 5, 10, 40, 80, and 120 ppm with a correlation coefficient (R^2) of 0.9997. These results indicate an excellent linear relationship between concentration and peak area for both analytes within the studied range.

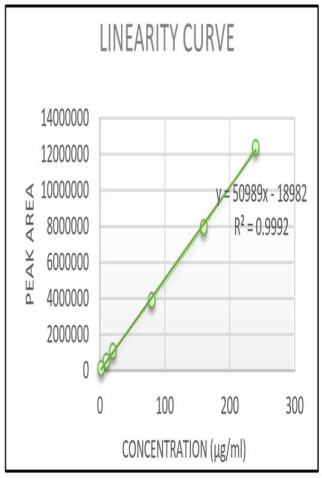

Figure No. 9: Linearity graph of Alfuzosin

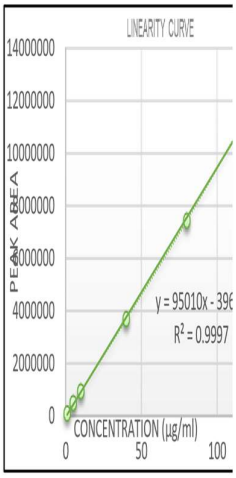

Figure No. 10: Linearity graph o

Repeatability and Precision: Repeatability was evaluated by six replicate injections (n = 6) of the standard solution (100 ppm Alfuzosin and 50 ppm Tadalafil), and %RSD was found within acceptable limits. Precision was assessed at 80%, 100%, and 120% levels using standard solutions of 80, 100, and 120 ppm of Alfuzosin and 40, 50, and 60 ppm of Tadalafil for intraday and interday studies.

Table No. 2: Repeatability data for Alfuzosin and Tadalafil

Conc. (µg/ml) (TADA)	Peak area (µAU) (TADA)	Conc. (µg/ml) (ALFU)	Peak area (µAU) (ALFU)
50	4588167	**100**	4939835
50	4538301	**100**	4886207
50	4602061	**100**	4967874
50	4552745	**100**	4958472
50	4568474	**100**	4971254
50	4599872	**100**	4899285
Mean ± SD	4574937 ± 26111.11	**Mean ± SD**	4937155 ± 36325.83
RSD	0.570	**RSD**	0.735

Table No. 3: Inter day Intra day precision data for Alfuzosin and Tadalafil

Level	Inter day precision (Alfuzosin)		Intra day precision (Alfuzosin)	
	Mean peak area ± SD	**% RSD**	**Mean peak area ± SD**	**% RSD**
80%	3957276 ± 7803.63	0.197	3958200 ± 6500.45	0.164
100%	4959732 ± 11515.23	0.232	4960500 ± 9500.75	0.192
120%	5773841 ± 4915.80	0.085	5774600 ± 4700.60	0.081
Level	**Inter day precision (Tadalafil)**		**Intra day precision (Tadalafil)**	
	Mean peak area ± SD	**% RSD**	**Mean peak area ± SD**	**% RSD**
80%	3747899 ± 10351.34	0.276	3748100 ± 9200.50	0.245
100%	4610966 ± 12593.57	0.273	4610500 ± 10900.00	0.236
120%	5450912 ± 7157.335	0.131	5431200 ± 6100.75	0.112

Accuracy: Accuracy was evaluated at 80%, 100%, and 120% levels by appropriate dilution of the standard stock solution with acetonitrile to obtain concentrations of 180, 200, and 220 ppm of Alfuzosin and 90, 100, and 120 ppm of Tadalafil, respectively. The percentage recovery was determined for each level.

Table No. 4: Accuracy studies data for Alfuzosin and Tadalafil

Conc. Level	Amount added (mg) ALFU	Amount added (mg) TADA	Amount Recovered (µg/ml) (ALFU)	Amount Recovered (µg/ml) (TADA)	% Recovery	
					ALFU	TADA
NA	100	50	NA	NA	NA	NA
80%	80	40	80.113	39.988	100.14	99.97
100%	100	50	101.762	49.933	101.76	99.87
120%	120	60	122.187	60.825	101.82	101.37

Robustness: The robustness of the developed method was evaluated by introducing small deliberate variations in flow rate, mobile phase composition, and temperature. Changes in flow rate (0.5 and 0.7 mL/min), mobile phase ratio (67:33 and 63:37 buffer:ACN), and temperature conditions resulted in minor variations in retention time; however, the assay values for both Alfuzosin and

Tadalafil remained within acceptable limits with low %RSD. These results indicate that the method is robust and not significantly affected by small changes in chromatographic conditions.

Assay: The developed RP-HPLC method was successfully applied for the assay of Alfuzosin and Tadalafil in marketed tablet dosage form. The assay results were found to be 101.40 for Tadalafil and 101.10 for Alfuzosin which is within the acceptable pharmacopeial limits, indicating good accuracy and applicability of the method for routine quality control analysis.

LOD and LOQ: The sensitivity of the developed method was evaluated in terms of limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values for Alfuzosin were 0.04 and 0.06 respectively and for Tadalafil were 0.07 and 0.09 respectively which was found to be sufficiently low, demonstrating the high sensitivity of the proposed RP-HPLC method.

Statistical Comparison: The statistical comparison between the developed and reported methods was carried out using one-way ANOVA, considering recovery as the analytical parameter. The study was performed in triplicate (n = 3) at a significance level of $\alpha = 0.05$. The calculated F-value was found to be lower than the critical F-value at the 95% confidence level, and the p-value was greater than 0.05, indicating that the observed variation between the two methods was not statistically significant and remained within acceptable analytical variability. Therefore, it can be concluded that there is no significant difference between the developed and reported methods.

CONCLUSION

The developed RP-HPLC method was successfully validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for the simultaneous estimation of Tadalafil and Alfuzosin. The method exhibited excellent linearity over the concentration range of 1–120 µg/mL for Tadalafil and 2–240 µg/mL for Alfuzosin. Accuracy studies showed satisfactory percentage recoveries in the range of 99.87– 101.37% for Tadalafil and 100.14–101.82% for Alfuzosin, confirming the accuracy of the method. Intermediate precision studies demonstrated %RSD values of less than 2%, indicating good precision and reproducibility. The specificity study confirmed that there was no co-elution with the main peaks, demonstrating the selective nature of the method. The low LOD values of 0.05 µg/mL for Tadalafil and 0.03 µg/mL for Alfuzosin, along with LOQ values of 0.07 µg/mL and 0.05 µg/mL, respectively, indicated the high sensitivity of the developed method.

Overall, the method was found to be accurate, precise, specific, and sensitive for routine quality control analysis of both drugs in pharmaceutical dosage forms.

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