Stability Indicating Analyticalmethod Development and Validation of Alfuzosin Hydrochloride and Tadalafil by Using Rp-Hplc

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***Abstract*—A simple, accurate, sensitive, and repeatable reverse phase high performance liquid chromatography (RP-HPLC) method has been created to measure s amount of Tadalafil and Alfuzosin HCl in medicinal dosage form. For the chromatographic separation of Tadalafil and Alfuzosin HCl on the Waters Alliance-e2695, a Luna Phenyl Hexyl (250x 4.6mm, 5µ) column was used, and the mobile phase was made up of 30:70% v/v Acetonitrile, Ammonium acetate pH-3.0, and OPA. The flow rate was 1 ml/min, and a photodiode array detector working at room temperature was used for absorption at 294nm. In the case of Tadalafil, the number of potential plates was NLT 2000, and the tailing factor should not be more than 2. It is always less than 2.0 for the relative standard deviation of peak areas across all measures. Following ICH guidelines, the suggested method was proven to work. It was discovered that the method was easy, cheap, accurate, exact, and strong for studying the stability of Tadalafil and Alfuzosin HCl in large amounts.**

***Index Terms*—HPLC, Tadalafil, Alfuzosin HCl,specificity, purity and ICH guidelines. ABBREVATIONS: HPLC-High performance liquid chromatography, HCL-Hydrochloric Acid**

1. INTRODUCTION

A frequent prescription drug for male patients with benign prostatic hyperplasia (BPH) is alfuzosin hydrochloride, an alpha-adrenergic antagonist. It works by causing the smooth muscle tissue at the neck of the bladder and the prostate gland to relax. Increased urine flow and a decrease in the symptoms commonly linked to BPH are the results of this effect. The drug's mechanism is specifically targets and inhibits the lower urinary system's alpha-1 adrenergic receptors.

The main purpose of the prescription medication tadalafil is to treat male erectile dysfunction (ED). It is categorised as an inhibitor of phosphodiesterase type 5 (PDE5). When sexually aroused, men can get and maintain an erection because to the medication's enhancement in penile blood flow. The medicine works by inhibiting the PDE5 enzyme, which raises cyclic guanosine monophosphate (cGMP) levels and improves blood circulation. supported by important health agencies, such as the FDA and EMA. Tadalafil and alfuzosin hydrochloride both treat the symptoms of ED and BPH, and they may have complementing benefits on reducing LUTS. may provide better overall symptom alleviation than medications used alone.

For the separation, identification, and quantification of molecules, the pharmaceutical sector frequently uses high-performance liquid chromatography (HPLC), a crucial analytical method.

2. MATERIALS AND METHODS

2.1The materials (Apparatus & chemicals) were tabulated inTable:1, Table:2.
2.2 chromatographic conditionswere tabulated in Table:3.
Based on the above chromatographic conditions the optimized method was fixed**.** Table no:4, FigNo:1
2.3. Preparations of standard solution
Accurately weigh and transfer 5 mg of Tadalafil and 10 mg of Alfuzosin HCl working standards to another 10 ml clean dry volumetric flask. Add Diluent and sonicate to thoroughly dissolve, then make volume up to the mark with the same solvent. Pipette 5ml of the

aforementioned tadalafil solution into a 10ml volumetric flask and fill to the mark with diluent. (Stock Solution)

2.3.1. Preparation of Working standard solution

Further pipette 1 ml of the aforesaid stock solutions into a 10 ml volumetric flask and dilute with diluent to the mark. (25ppm Tadalafil, 100ppm Alfuzosin HCl)

2.4. Analytical Method Validation

2.4.1. System Suitability:

Tailing factor for the peaks due to Standard solution should not be more than 2.0 Theoretical plates for the Standard solution should not be less than 2000. The resolution for the peaks of standard solution must be no less than 2.

2.4.2. Specificity:

The specificity of an analytical technique refers to its capacity to accurately measure the analyte of interest without interference from the blank or known contaminants. To this end, a blank chromatogram, a standard chromatogram, and a sample chromatogram were documented. The chromatogram of the blank exhibits no reaction at the retention times of the medications, so confirming the specificity of the drug responses.

2.4.3. Linearity:

The method's capacity to produce findings directly proportionate to the analyte concentration within a specified range. Construct calibration curves by examining samples with known concentrations (usually 5–6 levels) and graph the response against concentration. Determine the correlation coefficient (R^2), which should be no less than 0.99 for the majority of cases.

2.4.4. Range:

The range of an analytical method is the interval between the upper and lower concentrations of an analyte, inclusive of these values, that have been validated for precision, accuracy, and linearity.

Acceptance Criteria**:** Correlation coefficient should be not less than 0.999.

2.4.5. Accuracy

Accuracy is defined as the degree of agreement between the reference or theoretical concentration (true value) and the measured concentration (obtained value). The usual solutions include Accuracy -50%, Accuracy -100%, and Accuracy -150% solutions.

% Recovery = (Measured concentration/Theoretical concentration) × 100

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

2.4.6. Precision

Precision refers to the extent of reproducibility of an analytical process under standard operational conditions. There are three sorts of precision.

1. System precision
2. Method precision
3. Intermediate precision (a. Intraday precision, b. Inter day precision)

The precision of the system is verified using standard chemical substances to confirm the proper functioning of the analytical system. The percentage of the drug in this peak area from six determinations must be quantified, and the percentage relative standard deviation (RSD) should be determined.

In method precision, a homogeneous sample from a single batch should be analysed six times. This denotes whether a method yields consistent results for a singular batch. Analyse the sample six times and obtain the percentage relative standard deviation (% RSD).

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 25ppm of Tadalafil, 100ppm of Alfuzosin HCl).

Acceptance Criteria**:** The % RSD for the absorbance of six replicate injections results should not be more than 2%.

2.4.7. Robustness:

To assess the method's robustness, intentional alterations were made to the flow rate, mobile phase composition, and temperature variation.

a. The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 25 ppm of Tadalafil and 100 ppm of Alfuzosin HCl were produced and evaluated using various flow rates in conjunction with the method flow rate. Upon evaluating the results mentioned earlier, it can be determined that the difference in flow rate greatly impacted the approach. Thus, it demonstrates that the approach remains strong despite a variation in flow rate of ±10%.

b. The variation of Organic Phase ratio.

A standard solution of 25 ppm Tadalafil and 100 ppm Alfuzosin HCl was prepared and analysed using varying mobile phase ratios.

2.4.8. Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

LOD = 3.3 X σ /S

LOQ = 10 X σ /S

2.5. DEGRADATION STUDIES:

Acid degradation:

Pipette 1 ml of the specified solution was introduced into a 10 ml vacuum flask, succeeded by the addition of one millilitre of 1N HCl. The vacuum flask was subsequently held at 60°C for one hour prior to neutralisation with 1 N NaOH and dilution to 10 ml with a diluent. Utilise 0.45-micron syringe filters to purify the solution and thereafter transfer it to bottles.

Alkali degradation:

Pipette 1 ml of the above solution into a 10 ml volumetric flask and add 1 ml of 1N NaOH. The volumetric flask was maintained at 60ºC for one hour, thereafter neutralised with 1N HCl, and diluted to a final volume of 10 mL with a diluent. Utilise 0.45-micron syringe filters to filter the solution and transfer it into vials.

Thermal degradation

A sample of Tadalafil and Alfuzosin HCl was placed in a petri dish and incubated in a hot air oven at 105°C for 3 hours. The sample was subsequently diluted using diluents and injected into HPLC for analysis.

Peroxide degradation

pipette 1 ml of the stock solution was introduced into a 10 ml vacuum flask, followed by the addition of one ml of 3 percent w/v hydrogen peroxide, and the volume was adjusted to the mark using a diluent. The vacuum flask was thereafter held at 60°C for one hour. The vacuum flask was thereafter maintained at room temperature for 15 minutes. Utilise 0.45-micron syringe filters to filter the solution and subsequently transfer it to bottles.

Reduction degradation

Pipette 1 ml of the stock solution mentioned earlier was introduced into a 10 ml vacuum flask, followed by the addition of 1 ml of 10% sodium bisulfite, and the total volume was adjusted to the desired level using diluent. The vacuum flask was thereafter held at 60°C for one hour. The vacuum flask was thereafter maintained at room temperature for 15 minutes. Utilise 0.45-micron syringe filters to purify the solution and subsequently transfer it into bottles.

Photolytic degradation

A sample of Tadalafil and Alfuzosin HCl was subjected to a photostability chamber for a duration of three hours. The sample was subsequently diluted using diluents, injected into HPLC, and analysed.

Hydrolysis degradation

Pipette One ml of the aforementioned stock solution was introduced into a 10 ml vacuum flask, followed by the addition of 1 ml of HPLC-grade water, and the volume was adjusted to the desired level with diluent. The vacuum flask was thereafter held at 60°C for one hour. The vacuum flask was thereafter maintained at room temperature for 15 minutes. Utilise 0.45-micron syringe filters to filter the solution and subsequently transfer it to bottles.

3. RESULTS AND DISCUSSION

3.1. System Suitability:

The tailing factor for the peaks of Tadalafil and Alfuzosin HCl in the standard solution must not exceed 2.0. The theoretical plates for the peaks of Tadalafil and Alfuzosin HCl in the standard solution must not be lesser than 2000.

The resolution for the peaks of Tadalafil and Alfuzosin HCl in the standard solution must be no less than 2.

Conclusion: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

System suitability values were tabulated inTable No:5

3.2. Precision

Method precision

The %RSD (Relative Standard Deviation) for Alfuzosin HCl and Tadalafil was determined using six replicate injections. The average area for Alfuzosin HCl was 3,062,968, with a standard deviation of 12,751.220, and a %RSD of 0.42%. The mean area for Tadalafil was 763,695, with a standard deviation of 1,751.097, resulting in a %RSD of 0.23%. The low %RSD values signify great precision and repeatability of the procedure for both compounds.

Intermediate precision (Day-Day Precision)

Intermediate precision for Alfuzosin HCl and Tadalafil determined following six replicate injections. Day 1: The average area for Alfuzosin HCl

was 3,056,305, with a standard deviation of 18,177.320, yielding a %RSD of 0.59%. Day 2: The average area for Alfuzosin HCl was 3,060,129, with a standard deviation of 9,176.466, yielding a %RSD of 0.30%. Day 1: The mean area of Tadalafil was 763,735, with a standard deviation of 1,721.003, resulting in a %RSD of 0.23%. On Day 2, the mean area of Tadalafil was 765,138, with a standard deviation of 2,060.754, resulting in a %RSD of 0.27%. The results provide excellent intermediate precision for both analytes, reflecting the method's reliability when evaluated under varying conditions or over multiple days.

3.3. Accuracy

Samples for three accuracy levels were created via the conventional addition method. Triplicate injections were administered at each accuracy level, yielding mean recoveries of 99.63% for Alfuzosin HCl and 100.16% for Tadalafil.

3.4. Sensitivity

The Limit of Detection (LOD) for Alfuzosin HCl was 0.60 µg/ml, whereas the Limit of Quantitation (LOQ) was 2.00 µg/ml. The limit of detection (LOD) for teneligliptin was 0.15 µg/ml, whereas the limit of quantification (LOQ) was 0.50 µg/ml. These values demonstrate the method's capacity to detect and quantify low quantities of both analytes with great sensitivity.

3.5. Robustness

Robustness Results of Tadalafil by RP-HPLC: Table No:7

Flow Rate Change (ml/min):

- 0.9 mL (Less Flow): Retention time: 2.814 min, Peak area: 744269, Tailing: 1.18, Plate count: 15339, %RSD: 0.21
- 1.0 mL (Actual Flow): Retention time: 2.615 min, Peak area: 765354, Tailing: 1.15, Plate count: 15426, %RSD: 0.21
- 1.1 mL (More Flow): Retention time: 2.543 min, Peak area: 778524, Tailing: 1.12, Plate count: 15577, %RSD: 0.25

Organic Phase Change:

- 27:73 (Less Organic): Retention time: 2.938 min, Peak area: 731268, Tailing: 1.14, Plate count: 15204, %RSD: 0.32
- 30:70 (Actual): Retention time: 2.613 min, Peak area: 762874, Tailing: 1.11, Plate count: 15458, %RSD: 0.21
- 33:67 (More Organic): Retention time: 2.333 min, Peak area: 793257, Tailing: 1.07, Plate count: 15605, %RSD: 0.21

Robustness Results of Alfuzosin HCl by RP-HPLC

Flow Rate Change (ml/min):

- 0.9 mL (Less Flow): Retention time: 4.083 min, Peak area: 2967452, Resolution: 4.76, Tailing: 1.03, Plate count: 11534, %RSD: 0.31
- 1.0 mL (Actual Flow): Retention time: 3.921 min, Peak area: 3067743, Resolution: 5.12, Tailing: 0.98, Plate count: 11639, %RSD: 0.32
- 1.1 mL (More Flow): Retention time: 3.784 min, Peak area: 3233621, Resolution: 4.44, Tailing: 0.94, Plate count: 11756, %RSD: 0.60

Organic Phase Change:

- 27:73 (Less Organic): Retention time: 4.102 min, Peak area: 2817416, Resolution: 4.41, Tailing: 0.99, Plate count: 11469, %RSD: 0.72
- 30:70 (Actual): Retention time: 3.920 min, Peak area: 3053793, Resolution: 5.18, Tailing: 0.95, Plate count: 11612, %RSD: 0.32
- 33:67 (More Organic): Retention time: 3.612 min, Peak area: 3361411, Resolution: 4.85,
- Tailing: 0.91, Plate count: 11813, %RSD: 0.21

3.6. Linearity:The calibration curves were obtained by analysing samples of known concentrations (typically 5–6 levels) and plot the response vs. concentration. Calculated the correlation coefficient (R^2). see Table no.6.FigNo:2, FigNo:3.

3.7. specificity:blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drugs was specific**.** FIGNo:4, FIGNo:5, FIGNo:6.

4.CONCLUSION

The HPLC method developed to estimate particular drugs is simple rapid, reliable, precise, robust, and accessible. The mobile phase and solvents are cheap, reliable, sensitive, less time-consuming, and easy to prepare. The sample recoveries were in good agreement with their individual label claims and they indicated non-interference of formulation recipients in the estimation and can be utilised in laboratories for the routine analysis of selected medications. It is deduced that the simple and short proposed methods

be most useful for analysis purpose since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well. The present work found that RP-HPLC's stability indicating assay method was easy, accurate, precise, and specific and had no interference with the placebo and degradation products. Therefore, these can be employed for regular Tadalafil and Alfuzosin HCl analysis.

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LIST OF FIGURES

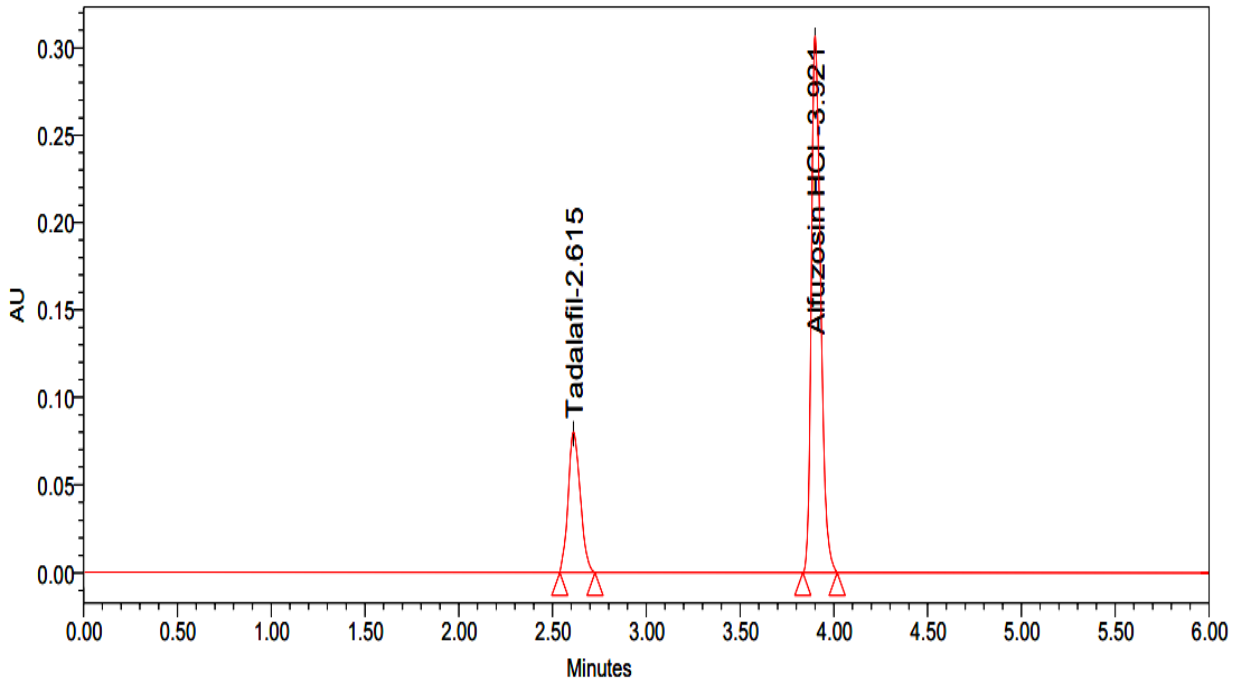


FIG.NO.1 Optimized chromatogram

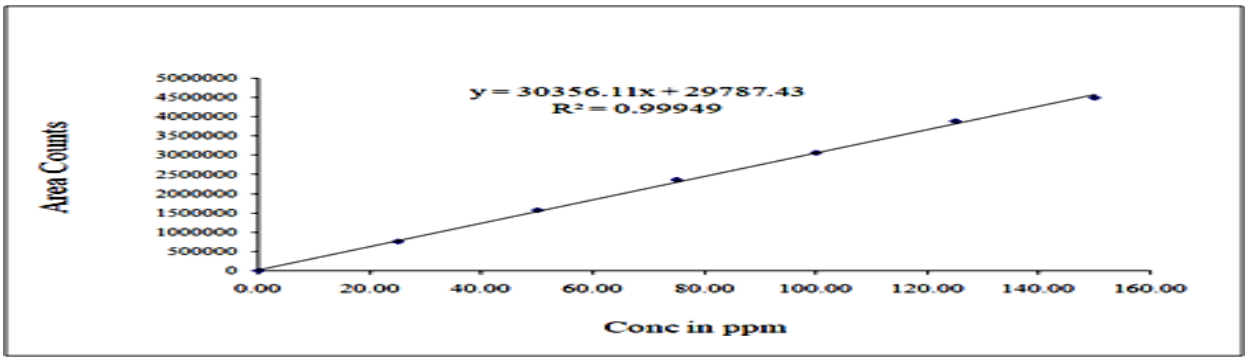


FIG NO:2. Calibration curve for Alfuzosin HCl

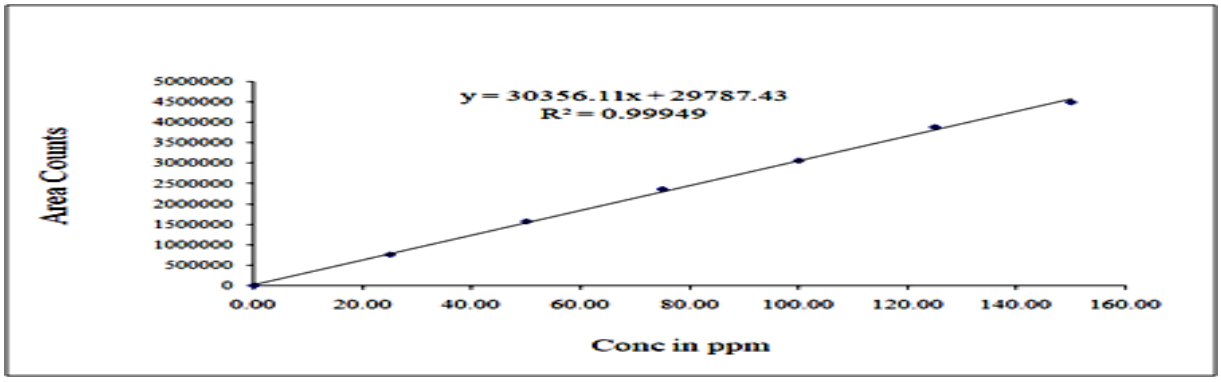


FIG NO:3. Calibration curve for Tadalafil

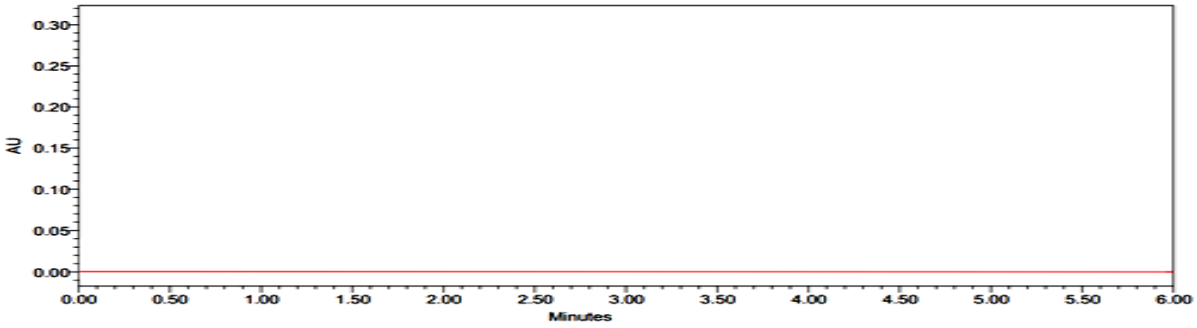


FIG NO:4. Chromatogram of blank

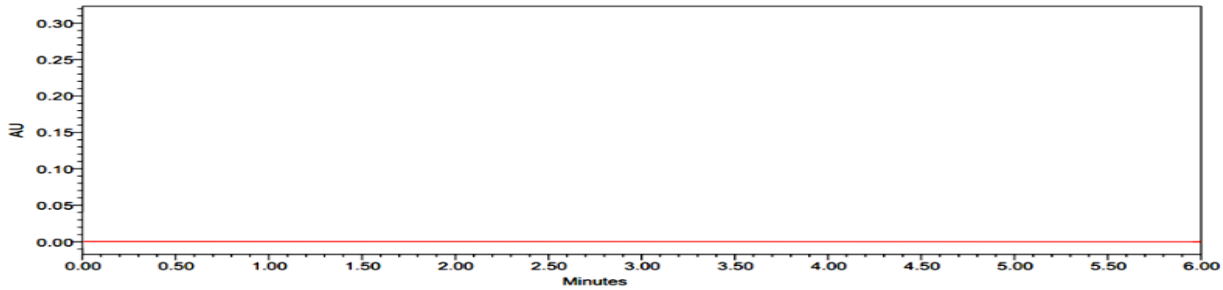
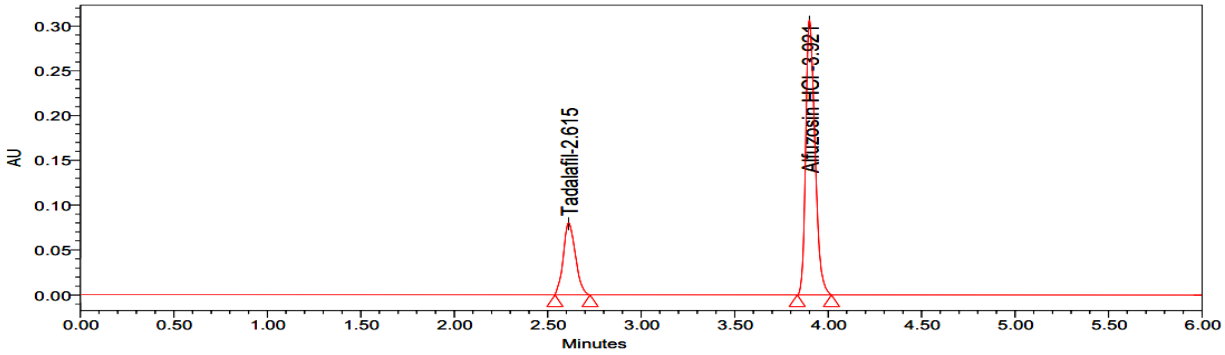


FIG NO:5. Chromatogram of placebo



FIGNO:6. sample chromatogram

LIST OF TABLES:

Table No.1: Apparatus used in HPLC

Sl. No	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters e 2695- Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	Pipettes, beakers and Burettes	-	Borosil
5.	Ultrasonicator	UCA 701	Unichrome
6.	Pump	Isocratic model	--

Table No.2: chemicals used in HPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Merck
2.	Water (Milli Q)	HPLC	In house production
3.	Ammonium acetate	AR	Merck
4.	Ortho Phosphoric acid	AR	Merck

Table No.3: Optimized Chromatographic conditions

Instrument used	Waters HPLC with auto sampler and PDA detector.
Column	Luna Phenyl Hexyl (250x 4.6mm, 5µ)
Mobile phase ratio	Acetonitrile: Ammonium acetate pH-3.0/OPA (30:70)
Detection wavelength	294nm
Flow rate	1ml/min
Injection volume	10µl
Run time	6min
Mode of separation	Isocratic mode
Temperature	Ambient (25° C)
Observation	This method is suitable for validation

Table No.4: Results for Optimized conditions

Sr. No	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Tadalafil	2.615	765354	15426	1.15	
2	Alfuzosin HCl	3.921	3067743	11639	0.98	5.12

Table No.5: System suitability parameters for Tadalafil & Alfuzosin HCl

S.no	Parameter		Tadalafil	Alfuzosin HCl
1	Retention time		2.615	3.921
2	Plate count		15426	11639
3	Tailing factor		1.15	0.98
4	Resolution		----	5.12
5	%RSD		0.21	0.32

Table No.6: Results of linearity for Tadalafil & Alfuzosin HCl

S.NO	Tadalafil		Alfuzosin HCl	
	Conc. µg/ml	Peak area	Conc. µg/ml	Peak area
1	6.25	197381	25.00	758753
2	12.50	387739	50.00	1571560
3	18.75	572045	75.00	2362175
4	25.00	765437	100.0	3066503
5	31.25	952224	125.0	3887600
6	37.50	1119632	150.0	4498881
Regression equation	y = 29978.74x +8535.43		y =20924.88x + 21904.04	
Slope	29978.74		30356.11	
Intercept	8535.43		29787.43	
R^2	0.99981		0.99949	

Table No.7: Degradation Studies

Results: % Degradation results	Tadalafil					Alfuzosin HCl				
	Area	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	764248	100	0	4.132	9.674	3069823	100	0	7.921	15.224
Acid	671562	87.9	12.1	4.154	9.687	2683017	87.4	12.6	7.955	15.298
Alkali	674509	88.2	11.8	4.175	9.612	2667075	86.9	13.1	7.943	15.214
Peroxide	653231	85.5	14.5	4.169	9.653	2579507	84.0	16.0	7.913	15.235
Reduction	694144	90.8	9.2	4.107	9.625	2724461	88.7	11.3	7.948	15.269
Thermal	760123	99.4	0.6	4.132	9.697	3050156	99.3	0.7	7.924	15.278
Photolytic	741387	97.0	3.0	4.141	9.658	3018792	98.3	1.7	7.928	15.205
Hydrolysis	746556	97.7	2.3	4.187	9.661	3030408	98.7	1.3	7.934	15.277