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GREEN RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TAMSULOSIN AND TADALAFIL WITH GREENNESS ASSESSMENT

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Abstract

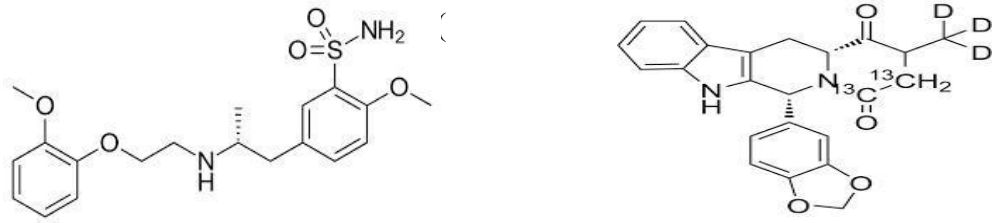
A streamlined, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and comprehensively validated for the simultaneous determination of Tamsulosin and Tadalafil in pharmaceutical dosage forms. Separation was achieved on a Platisil C18-SB column (150 × 4.6 mm, 3 µm) using an isocratic mobile phase of 5 mM Ammonium Acetate: Methanol (70:30 v/v) at 0.8 mL/min, with UV detection at 243 nm. Peaks were well-resolved, with optimal retention times and no excipient interference. Adhering strictly to ICH Q2(R1) guidelines, the method displayed linearity (R^2 >0.999) over 1.6–8 µg/mL for Tamsulosin and 20–100 µg/mL for Tadalafil. Precision was outstanding, as %RSD remained below 2% in repeatability studies. Accuracy was verified via recovery data (98–102%) at 50%, 100%, and 150% levels. Sensitivity excelled, with limits of detection at 0.4 µg/mL (Tamsulosin) and 1.3 µg/mL (Tadalafil), and limits of quantification at 1.3 µg/mL and 4.3 µg/mL, respectively. Specificity and robustness were also confirmed. The greenness of the developed method was assessed using AGREE software, and the method obtained a score of 0.74, indicating good environmental performance. This rapid, reliable RP-HPLC method is eminently suited for routine quality control of Tamsulosin-Tadalafil formulations.

1. INTRODUCTION:

Tamsulosin and tadalafil are frequently used in the management of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH), especially in men who also

experience erectile dysfunction (ED). Chemical structures of tamsulosin and tadalafil are shown in Figure 1(a&b). Tamsulosin, a selective α_1A-adrenoceptor antagonist, induces relaxation of prostatic and bladder-neck smooth muscle, which improves urinary flow and alleviates obstructive symptoms. Tadalafil, a phosphodiesterase-5 (PDE5) inhibitor shown a chemical, augments nitric oxide–driven accumulation of cyclic guanosine monophosphate (cGMP), resulting in vasodilation, smooth-muscle relaxation, enhanced erectile capacity, and a modest improvement in urinary symptoms. When administered in combination, these agents exert complementary and synergistic effects, and fixed-dose products containing both drugs have been approved by regulatory bodies such as the US FDA for the treatment of BPH with or without ED. In accordance with guidance from the International Council for Harmonisation (ICH), analytical procedures for such dosage forms must be fully validated to confirm their reliability, including assessment of accuracy, precision, specificity, robustness, and system suitability criteria.

Quantifying tamsulosin and tadalafil together in a single formulation is analytically demanding due to their distinct physicochemical characteristics and different concentration levels. Reverse-phase high-performance liquid chromatography (RP-HPLC) is well suited for this purpose because of its resolving power, sensitivity, and reproducibility. The present study describes the development and validation of a straightforward, reliable RP-HPLC method for the simultaneous determination of both compounds in pharmaceutical preparations, conforming to ICH recommendations, to facilitate routine quality-control and stability-evaluation of the combination product.



(a) (b)

Figure1: Chemical Structure A) Tamsulosin and B) Tadalafil

2. EXPERIMENTAL

2.1 Materials:

All chemicals and reagents employed in the study were of HPLC grade. Triethylamine (TEA), procured from Qualigens, was utilized in the estimation of Tamsulosin and Tadalafil. Formic acid of HPLC grade, also sourced from Qualigens, served the same purpose. High-purity water from Qualigens was used throughout the analysis of all drugs. Acetonitrile of HPLC grade, obtained from Qualigens, was likewise applied across the assays. Methanol of HPLC grade, supplied by Rankem, was employed for the estimation of all drugs.

2.2 Instruments:

The experimental study employed several analytical instruments. A Waters High Performance Liquid Chromatograph (HPLC) equipped with Empower Software, a 2695 Separation Module, and a 2487 UV Detector was used for chromatographic analysis. A UV/VIS Spectrometer (Lab India, Model UV 3000) facilitated wavelength determination. The pH meter (Adwa, Model AD 1020) was utilized to measure solution pH. An analytical balance (Afcoset, Model ER-200A) ensured precise weighing of chemicals and samples. Additionally, standard laboratory glassware, including pipettes, burettes, and beakers from Borosil, supported routine experimental procedures.

2.3 Preparation of Solutions:

Preparation of Ammonium Acetate Buffer:

To prepare Ammonium Acetate buffer solution by adding 0.3854 g in 1000ml HPLC water adjust the solution.

Preparation of mobile phase:

Mix a mixture of TEA above and MeOH and degas in ultrasonic water bath for 5 minutes. Filter through0.45 µ filter under vacuum filtration.

Stock solution of tamsulosin and tadalafil:

Accurately weigh and transfer 1.6mg of Tamsulosin and 20mg Tadalafil working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of working standard solution:

Accurately weighed and transferred 1.6 mg of Tamsulosin and 20 mg of Tadalafil working standards into a clean, dry 20 mL volumetric flask. Added sufficient quantity of diluent and sonicated to dissolve the contents completely. The volume was then made up to the mark with the same diluent

2.4 Methodology Method Development:

The greenness of the developed RP-HPLC method was assessed using AGREE software, confirming that the method is environmentally friendly and sustainable. A simple and reliable RP-HPLC method was developed for the simultaneous estimation of Tamsulosin and Tadalafil in a single run using a Waters HPLC system with Empower 2695 software and a 2487 UV detector, giving good separation in a short time with less use of organic solvents. During method development, important conditions like mobile phase composition, pH, and detection wavelength were carefully adjusted using a LABINDIA UV-3000 UV/Visible spectrophotometer and an Adwa AD-1020 pH meter to achieve better performance while keeping the method eco-friendly. All solutions were prepared accurately using Borosil glassware and an Accost ER-200A analytical balance to maintain consistency and reduce errors. The method used safer and optimized amounts of reagents such as potassium dihydrogen phosphate (KH_2PO_4), water, methanol, and acetonitrile, helping to reduce chemical waste and environmental impact. Overall, the AGREE results showed that the method is green, simple, and suitable for routine

pharmaceutical analysis.

Method Validation

The validation has been performed by following the ICH Q2 (R1) guidelines and parameters like system suitability, accuracy, precision, linearity, robustness, LOD, and LOQ have been evaluated.

System suitability:

System suitability characteristics like theoretical plates, retention time, tailing factor, and percentage RSD can be measured using the prepared standard solution injection six times.

Accuracy:

It refers to the closeness of a measured result to the actual result. Accuracy can be determined using the method of recovery.

Specificity:

By comparing the data from the drug solution before and after spiking, it was possible to conclude that the approach was specific because there was no discernible interference from the blank with the recovery of tamsulosin and tadalafil

Precision:

Precision is the measurement of the degree to which various outputs of the same quantity agree with one another. Evaluated on the same day and three different days in order to evaluate the intra-day and interday fluctuations. The percentage RSD of the peak area and retention time were calculated.

Linearity and range:

Accurately weighed and transferred 1.6 mg of Tamsulosin and 20 mg of Tadalafil working standards into a 20 mL clean, dry volumetric flask, added diluent, sonicated to dissolve completely, and made up the volume to the mark with the same diluent to obtain the stock solution.

LOD and LOQ:

Limit of Detection and Limit of Quantitation for the calculations that determined the limits of detection (LOD) and quantification (LOQ), the signal-to-noise ratio was set at 3:1 for detection and 10:1 for quantification.

Robustness

Robustness was assessed by slightly modifying the parameters of the approach and tracking the impact on the method through the results of system compatibility tests.

3.0 RESULTS AND DISCUSSIONS:

System Suitability

System suitability tests are conducted by injecting the prepared standard solution five times and measuring parameters such as theoretical plates, retention time, tailing factor, and %RSD. All the results shown in figure 2 and table 1.

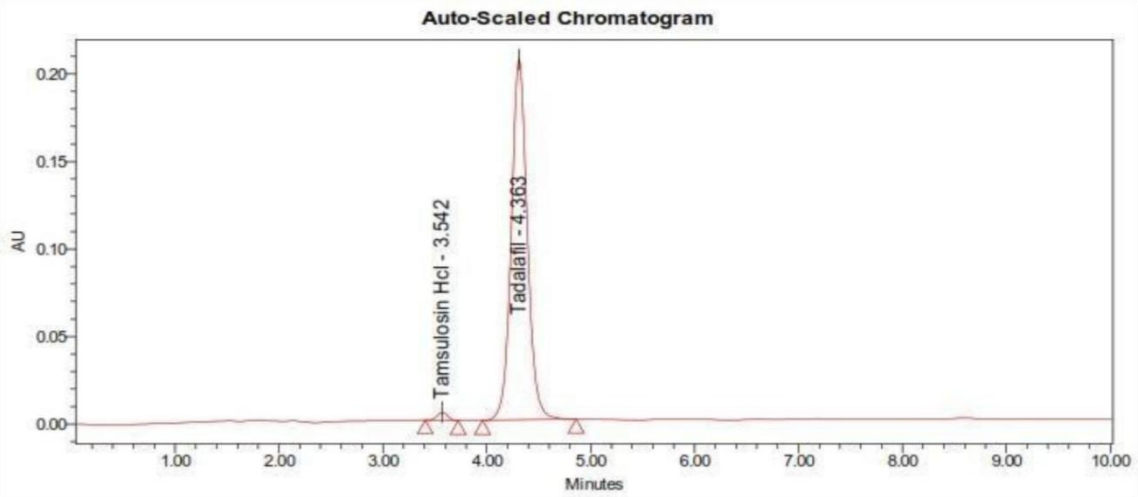


Fig 2. Optimized chromatogram of Tamsulosin and Tadalafil

Table 1: Results of system suitability parameters

S. No	Name	RT (min)	Area (µV sec)	Height (µV)	Resolution	Tailing Factor	Theoretical plate count
1	**Tamsulosin HCL**	3.557	10596	13536	4.7	1.03	3050
2	**Tadalafil**	4.337	522884	184612		1.07	6928

Specificity:

The specificity of the developed RP-HPLC method was evaluated by comparing chromatograms obtained from blank, standard, and sample solutions. No significant peak overlap or interference was detected at the retention times corresponding to tamsulosin and tadalafil, indicating that the method can effectively differentiate both drugs from formulation excipients and other matrix constituents. Representative chromatograms demonstrating the blank, standard, and sample analyses are displayed in Figures 3–4, respectively. A concise summary of the specificity findings is compiled in Table 2.

Table 2: Results table of Specificity

Parameter	Tamsulosin HCl	Tadalafil	Acceptance Criteria	Observation
Retention Time (min)	3.542 – 3.565	4.359 – 4.363	Should be consistent	Within limits
Resolution (Rs)	4.6 – 4.7 (vs Tadalafil)	4.6 – 4.7 (vs Tamsulosin)	≥ 2.0	Passed
Peak Purity	Pure (no co-eluting peaks)	Pure (no co-eluting peaks)	No interference	Passed
Interference from excipients	Not observed	Not observed	No interference	Passed
Baseline separation	Yes	Yes	Required	Achieved
Tailing factor	1.03 – 1.1	1.07 – 1.2	≤ 2	Passed

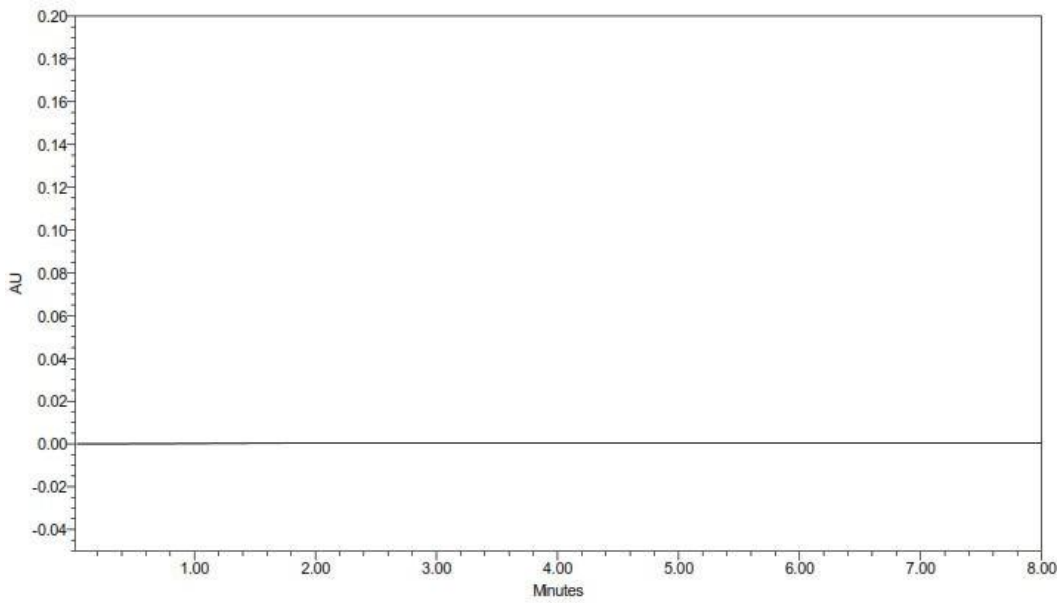


Fig 3. Chromatogram of Blank

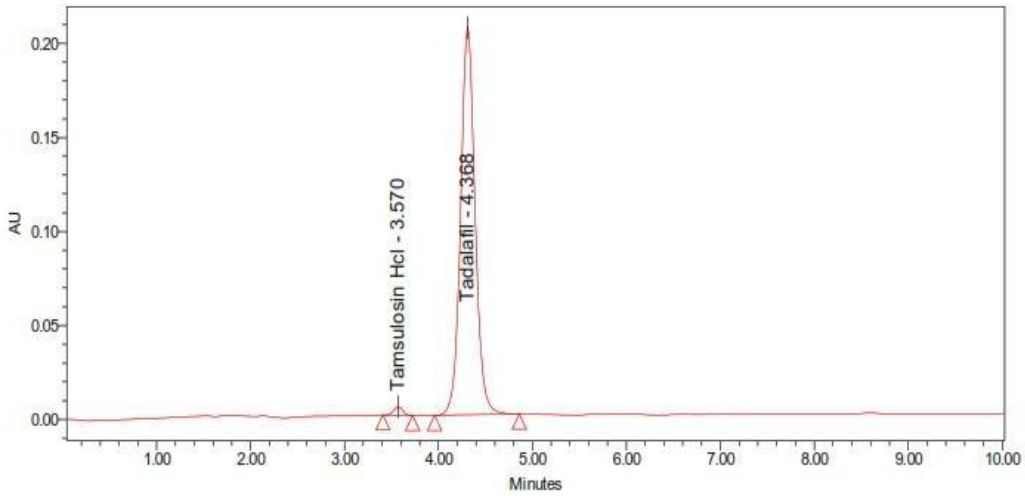


Fig 4. Chromatogram of Standard

Linearity:

Serial dilutions from the above stock were used to establish linearity ranges of 1.6–8 µg/mL for tamsulosin and 20–100 µg/mL for tadalafil. Calibration curves were generated by plotting peak area versus concentration for each analyte, yielding correlation coefficients (r^2) that indicated excellent linearity over the specified ranges. The linearity results are summarized in Table 3, and representative calibration plots are presented in Figure 5.

Table 3: Results of Linearity data for Tamsulosin HCL and Tadalafil

Sl.No	Tamsulosin		Tadalafil	
	Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
1	1.6	3274	20	152221
2	3.2	6748	40	336442
3	4.8	10596	60	522884
4	6.4	14192	80	705768
5	8	17384	100	881536

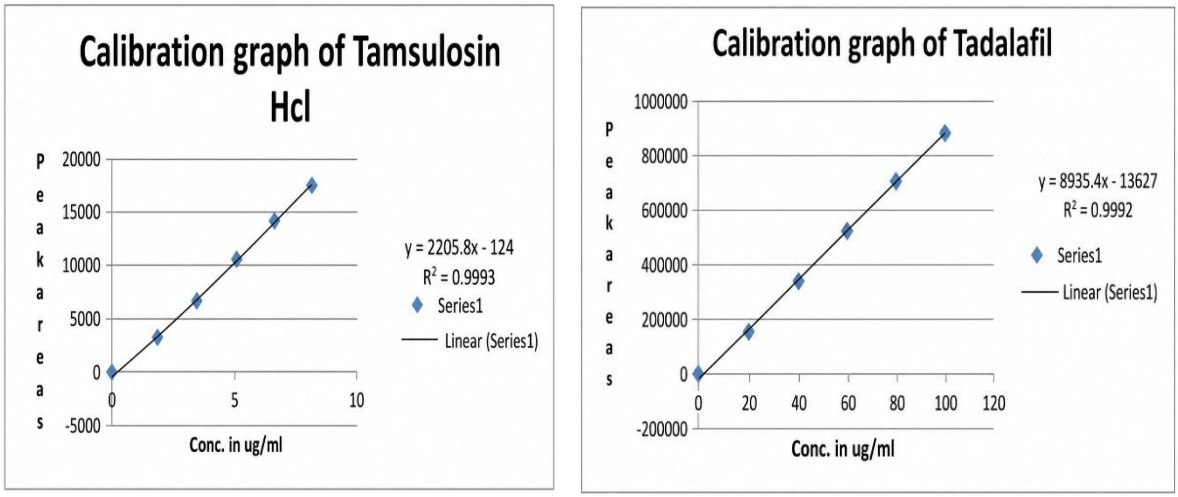


Fig 5. Calibration graph for Tamsulosin HCL and Tadalafil

Precision:

Accurately weigh 1.6 mg of Tamsulosin and 20 mg of Tadalafil working standards and transfer them into a clean, dry 20 mL volumetric flask. Add a suitable diluent, sonicate the mixture until both drugs are completely dissolved, and make up the volume to the mark with the same diluent to obtain the stock solution. From this stock solution, pipette 0.6 mL into a 10 mL volumetric flask and dilute to the mark with the diluent to prepare the final solution containing 4.8 ppm of Tamsulosin and 60 ppm of Tadalafil.

Table 4. Result of Precision for Tamsulosin HCL and Tadalafil

Injection	**Tamsulosin HCL area**		**Tadalafil area**	
	Intraday	**Interday**	**Intraday**	**Interday**
Injection 1	10553	10695	529784	526410
Injection 2	10452	10312	512752	512450
Injection 3	10498	10425	522255	529120
Injection 4	10327	10740	522363	521840
Injection 5	10678	10615	512125	513950
Injection 6	10771	10390	522486	525380
Average	10546.5	10529.5	520294.1667	521525.0
Standard	159.4976489	179.0327	6732.095823	6940.3474

deviation				
%RSD	**1.5**	**1.7**	**1.3**	**1.4**

Sensitivity Limit of Detection:

Accurately weigh 8 mg of Tamsulosin, dissolve in 25 ml diluent with sonication to prepare the stock solution, then dilute 1 ml and 1.25 ml portions separately to 10 ml with diluent to obtain the test solutions.

Table 4.a Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Conc.
Tamsulosin HCL	95	282	2.96	0.4 µg/ml
Tadalafil	135	400	2.96	1.3 µg/ml

Limit of Quantification:

Accurately weigh 8 mg of Tamsulosin and dissolve in a 25 ml volumetric flask with diluent, sonicating to volume for the stock solution; pipette 0.3 ml and 1.4 ml portions into separate 10 ml flasks and dilute to the mark. Similarly, weigh 25 mg of Tadalafil, dissolve in 25 ml diluent with sonication to prepare its stock solution, then pipette 1 ml into a 10 ml flask and dilute to the mark.

Table 4.b Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Conc.
Tamsulosin HCL	95	940	9.89	1.3 µg/ml
Tadalafil	135	1342	9.94	4.3 µg/ml

Accuracy:

Accurately weigh 1.6 mg of Tamsulosin and 20 mg of Tadalafil, dissolve in 20 ml diluent with sonication to prepare the stock solution, then dilute 0.6 ml to 10 ml to yield 4.8 ppm Tamsulosin and 60 ppm Tadalafil; for linearity, 50%, 100%, and 150% levels were prepared by dissolving 0.8/10 mg, 1.6/20 mg, and 2.4/30 mg of Tamsulosin/Tadalafil in 10 ml diluent flasks with sonication and appropriate dilution

.

Table 5. Accuracy (Recovery) data for Tamsulosin HCL and Tadalafil

%Concentration (at specification level)	Peak Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5279	0.8	0.79	99.27	99.05
100%	10612	1.6	1.58	98.90	99.05
150%	15763	2.4	2.38	99.00	99.05

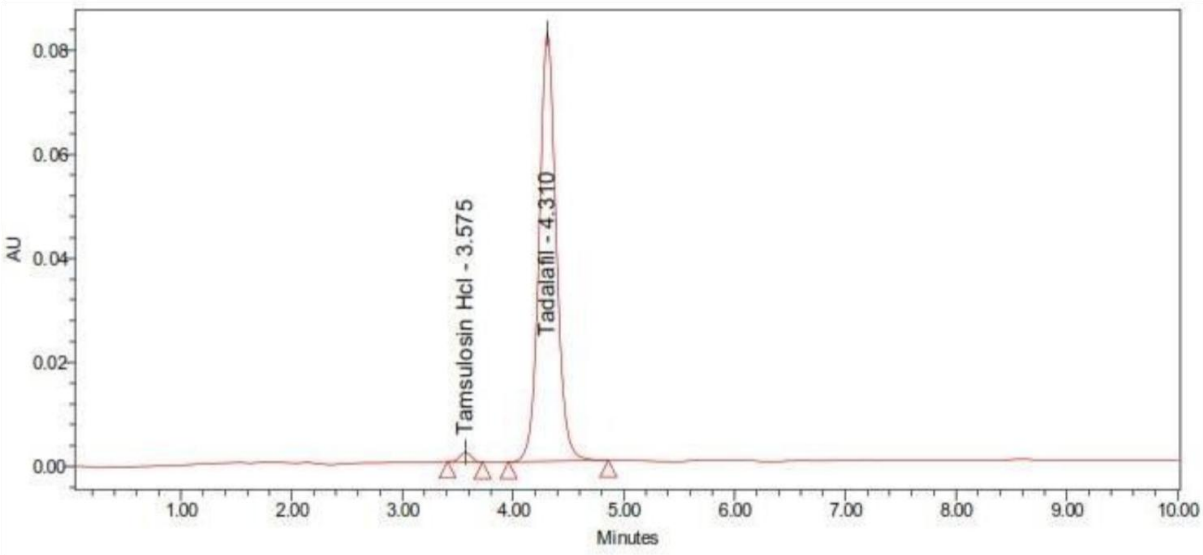


Figure 6. Chromatogram for Accuracy 50%

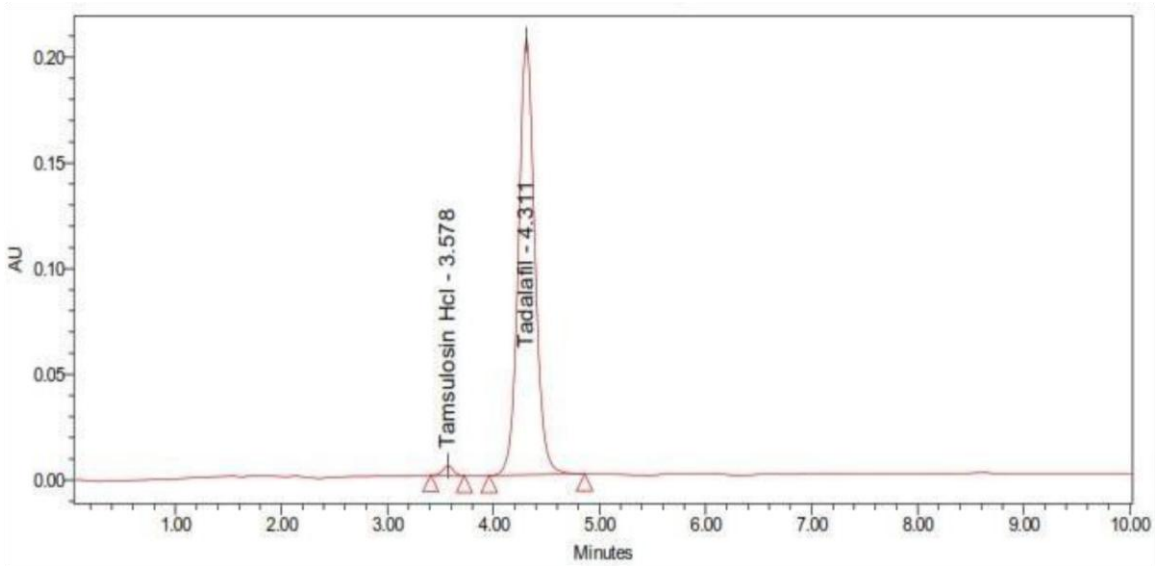


Figure 7 Chromatogram for Accuracy 100%

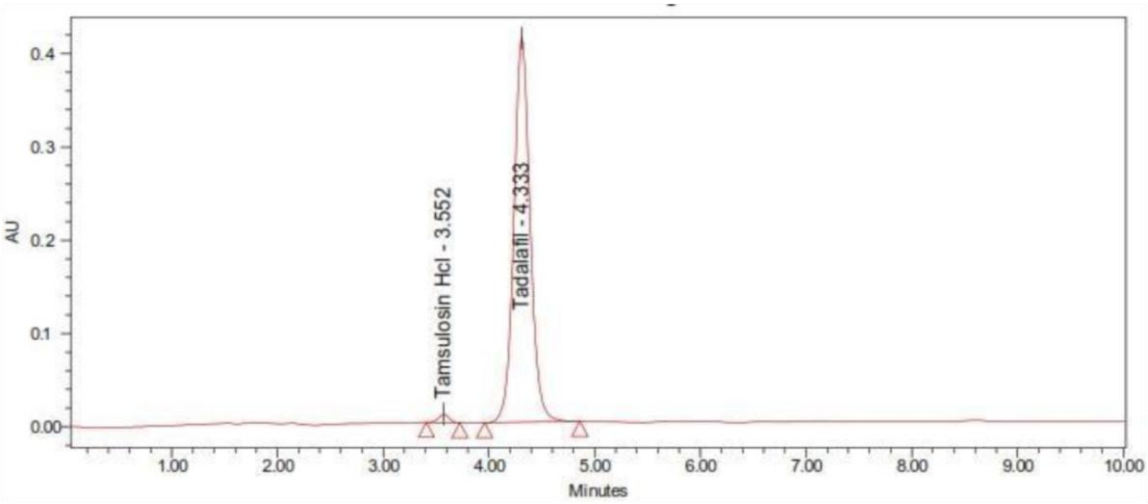


Figure 8: Chromatogram for Accuracy 150%

Robustness:

The chromatographic conditions were varied during the injection of both the standard and sample solutions of Tamsulosin HCl and Tadalafil. Despite these adjustments, the key performance parameters—including resolution, tailing factor, asymmetry factor, and theoretical plate count—remained consistent, showing no significant deviations.

Table 6. Results of Robustness studies for Tamsulosin HCL

S.NO	Flow rate (ml/min)	System suitability results of Tamsulosin HCL		System suitability results of Tadalafil	
		Theoretical plate count	Tailing Factor	Theoretical plate count	Tailing Factor
1	0.8	3042	1.03	6925	1.04
2	1.0	3050	1.03	6928	1.07
3	1.2	3054	1.01	6932	1.2

4.CONCULSION:

The validation of the analytical procedure for Tamsulosin HCl and Tadalafil unequivocally demonstrated its reliability and scientific rigor. The method exhibited excellent precision, accuracy, linearity, and robustness under the prescribed operating conditions. System suitability was firmly established, with critical parameters such as resolution, tailing factor, and theoretical plate count consistently meeting the acceptance thresholds. Assay validation confirmed

high recovery rates, underscoring the accuracy of quantification, while the correlation coefficient of 0.999 reflected outstanding consistency in linear response. Furthermore, the sensitivity of the method was reinforced by acceptable limits of detection and quantitation, ensuring its capability to detect and quantify even trace levels of analytes. Collectively, these findings affirm that the developed procedure is not only compliant with regulatory expectations but also highly dependable for routine analytical applications and advanced pharmaceutical investigations.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

We confirm that there are no conflicts of interest.

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