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Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Tamsulosin HCl and Tadalafil in Pharmaceutical Dosage Forms

Durgan Roopa[1], Dr. Naresh[2], Dr. Vijay Kumar Gampa*[3], B. Sravanthi[4]

[1]Department of Pharmaceutical Analysis, KGR Institute of Technology and Management, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad - 501302, Telangana, India.
[2]Associate Professor, Department of Pharmaceutical Analysis, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad -501302, Telangana, India.
[3]Principal and Professor, KGR Institute of Technology and Management, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad -501302, Telangana, India.
[4]Assistant Professor, Department of Pharmaceutical Analysis, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad -501302, Telangana, India.

A B S T R A C T

This research work outlines the optimization & validation of a HPLC method for the simultaneous analysis of Tamsulosin & Tadalafil. The optimized chromatographic conditions included the use of a Spursil C18-EP column with a MP composition of 70% per chloric acid & 30% ACN, a flow speed of 1.5 ml per min, & detection at 223 nm. SST confirmed that all parameters, including resolution (greater than 2), theoretical plates (above 2000), & tailing factor (below 2), were within acceptable limits. The assay results indicated a % assay of 99.90% for Tamsulosin& 101% for Tadalafil. The method exhibited linearity in the concentration extent of 1.6-8 µg per ml for Tamsulosin& 20-100 µg per ml for Tadalafil, with R2 exceeding 0.999. Precision, ID precision, & accuracy studies demonstrated low %RSD values (0.17% for Tamsulosin&0.14% for Tadalafil), indicating robustness &reliability. LOD were established at 0.026 µg per ml for Tamsulosin & 0.027 µg per ml for Tadalafil, while LOQ were 0.092µg per ml & 0.093 µg per ml, respectively. The technique proved resilient against variations in flow speeds& MP composition. Additionally, degradation studies assessed the stability of both compounds under various conditions, confirming their robustness. Overall, the validated HPLC technique is suitable for regular QC of Tamsulosin & Tadalafilin formulations.

Keywords: Tamsulosin, Tadalafil, %RSD, Detector, Analytical, RP-HPLC, C18-EP column

A R T I C L E I N F O

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C o n t e n t s

1. Introduction:

The pharmaceutical industry continually seeks to improve the quality and efficiency of drug development and validation processes. Analytical method development and validation are critical components of this process, ensuring that pharmaceutical products meet regulatory standards for efficacy, safety, and quality. High-Performance Liquid Chromatography (HPLC) is a preferred analytical technique due to its high resolution, sensitivity, and specificity. In particular, Reverse Phase HPLC (RP-HPLC) is widely utilized for its ability to separate compounds with varied polarities. This study focuses on the development and validation of a new RP-HPLC method for the simultaneous

estimation of Bisoprolol and Perindopril in their pure and pharmaceutical dosage forms, following the guidelines set forth by the International Council for Harmonisation (ICH).
Bisoprolol, a beta-blocker, is commonly used to manage hypertension, angina, and heart failure. It works by blocking beta-adrenergic receptors, reducing heart rate and myocardial contractility, thereby lowering blood pressure and decreasing the heart's oxygen demand. Perindopril, an angiotensin-converting enzyme (ACE) inhibitor, is also used to treat hypertension and heart failure. It functions by inhibiting the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, thus promoting vasodilation and lowering blood pressure. The combination of Bisoprolol and Perindopril provides a synergistic effect, making their simultaneous analysis in pharmaceutical formulations essential for quality control and therapeutic efficacy.
The development of an RP-HPLC method for these drugs involves the meticulous optimization of chromatographic conditions. This includes the selection of the mobile phase, column type, flow rate, and detection wavelength. The mobile phase must be carefully chosen to achieve optimal separation and resolution of the analytes. Common mobile phase components include water, methanol, and acetonitrile, often supplemented with buffer solutions to maintain pH stability. The choice of column, typically a C18 column, is crucial as it directly impacts the separation efficiency and resolution.

Once the chromatographic conditions are optimized, the method must be validated according to ICH guidelines. Validation is a comprehensive process that assesses various performance characteristics, including specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. Specificity ensures that the method can accurately identify and quantify Bisoprolol and Perindopril in the presence of other components, such as excipients and degradation products. Linearity evaluates the method's ability to produce results that are directly proportional to the analyte concentration over a specified range.

Accuracy and precision are vital parameters for ensuring that the method produces reliable and reproducible results. Accuracy is determined by comparing the test results with those obtained from a reference method or known standards, while precision is assessed by measuring the consistency of results from multiple analyses of the same sample. The LOD and LOQ are essential for determining the method's sensitivity, indicating the smallest amount of the analyte that can be reliably detected and quantified. Robustness testing evaluates the method's reliability under varying conditions, such as changes in pH, flow rate, and temperature, ensuring that the method remains consistent and accurate under different operational scenarios.

The successful development and validation of an RP-HPLC method for the simultaneous estimation of Bisoprolol and Perindopril have significant implications for pharmaceutical quality control. It ensures that these critical cardiovascular drugs meet the required standards of efficacy, safety, and quality, thereby enhancing patient outcomes and contributing to public health. Furthermore, this validated method can be applied in routine quality control, stability testing, and during the manufacturing process to ensure the consistent quality of pharmaceutical products.

In conclusion, the development and validation of a new RP-HPLC method for the simultaneous estimation of Bisoprolol and Perindopril represent a crucial advancement in pharmaceutical analysis. By following a systematic approach to method development and adhering to rigorous validation protocols as per ICH guidelines, this study aims to establish a robust, reliable, and accurate analytical method. This method will not only ensure compliance with regulatory standards but also contribute to the overall improvement of pharmaceutical analytical practices, thereby supporting the continued development of safe and effective cardiovascular therapies.

Drug Profile

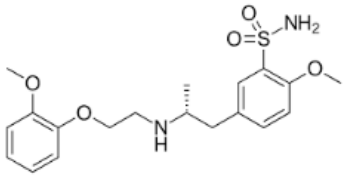

Figure.1. Tamsulosin

Basic Information
IUPAC Name: 5-[(2R)-2-[[2-(2-Ethoxyphenoxy) ethyl] amino] propyl]-2-methoxybenzenesulfonamide
Molecular Formula: $C_{20}H_{28}N_2O_5S$
Molecular Weight: 408.52 g/mol
Category: Alpha-1 adrenergic receptor blocker
Physical Properties
Melting Point: 234-238°C
pKa: 8.5 (basic)
Solubility
Solubility: Soluble in water, ethanol, and methanol.
Description
Tamsulosin is a medication used to treat the symptoms of benign prostatic hyperplasia (BPH), which is an enlarged prostate. It helps to relax the muscles in the prostate and bladder neck, making it easier to urinate12.
Mechanism of Action
Tamsulosin works by selectively blocking alpha-1 adrenergic receptors in the prostate and bladder neck, leading to relaxation of smooth muscle in these areas and improved urine flow.
Pharmacodynamics: Tamsulosin selectively blocks alpha-1 adrenergic receptors, which are found in the smooth muscle of the prostate and bladder neck.
Pharmacokinetics:
Absorption: Well absorbed from the gastrointestinal tract.
Distribution: Extensively bound to plasma proteins (94-99%).
Metabolism: Metabolized in the liver by CYP3A4 and CYP2D6.
Excretion: Primarily excreted in the urine.
Metabolism and Elimination

Metabolism: Metabolized in the liver by CYP3A4 and CYP2D6.
Route of Elimination: Primarily renal.
Protein Binding and Half-Life
Protein Binding: 94-99%
Half-Life: 9-13 hours12.
Uses: Treatment of benign prostatic hyperplasia (BPH).
Dosage: Typically 0.4 mg once daily, taken 30 minutes after the same meal each day12.

Side Effects

Common side effects include dizziness, runny or stuffy nose, and ejaculation problems. Serious side effects can include severe allergic reactions and orthostatic hypotension12.
Drug Interactions
Tamsulosin can interact with other alpha-blockers, PDE5 inhibitors, and medications that affect CYP3A4 and CYP2D6 enzymes12.
Storage: Store at room temperature, between 20°C to 25°C (68°F to 77°F), away from moisture and heat12.

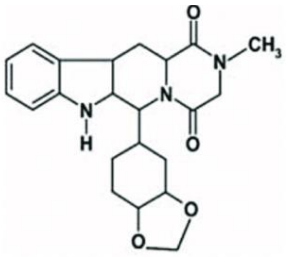

Figure.2 Taladafil

IUPAC Name: (6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydro-1H-pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione
Molecular Formula: $C_{22}H_{19}N_3O_4$
Molecular Weight: 389.41 g/mol
Category: Phosphodiesterase (PDE) inhibitor
Physical Properties
Melting Point: 298-300°C
pKa: 13.4 (basic)
Solubility
Solubility: Practically insoluble in water; soluble in ethanol and methanol.
Description
Tadalafil is a medication used to treat erectile dysfunction (ED), benign prostatic hyperplasia (BPH), and pulmonary arterial hypertension (PAH). It is known for its long duration of action, up to 36 hours12.

Mechanism of Action

Tadalafil works by inhibiting the enzyme phosphodiesterase type 5 (PDE5), which results in increased levels of cyclic guanosine monophosphate (cGMP). This leads to relaxation of smooth muscle and increased blood flow to specific areas of the body, such as the penis12.

Pharmacodynamics:

Tadalafil enhances the effect of nitric oxide by inhibiting PDE5, which is responsible for the degradation of cGMP in the corpus cavernosum.

Pharmacokinetics:

Absorption: Rapidly absorbed after oral administration.
Distribution: Widely distributed in body tissues.
Metabolism: Metabolized in the liver by CYP3A4.
Excretion: Primarily excreted in the feces (61%) and urine (36%).
Metabolism and Elimination
Metabolism: Metabolized in the liver by CYP3A4.
Route of Elimination: Primarily fecal and renal.
Protein Binding and Half-Life
Protein Binding: Approximately 94%
Half-Life: 17.5 hours12.
Uses and Dosage
Uses: Treatment of erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension.
Dosage:
Erectile Dysfunction: 10 mg prior to anticipated sexual activity, adjustable to 20 mg or 5 mg based on efficacy and tolerability.
BPH: 5 mg once daily.
PAH: 40 mg once daily12.
Side Effects: Common side effects include headache, dyspepsia, back pain, myalgia, nasal congestion, flushing, and limb pain. Severe side effects can include sudden vision loss, hearing loss, and priapism12.
Drug Interactions: Tadalafil can interact with nitrates, alpha-blockers, antihypertensives, and other medications that affect the CYP3A4 enzyme12.
Storage: Store at room temperature away from moisture and heat.

2. Methodology

Chemicals used:

For the estimation of Tamsulosin and Tadalafil, several chemicals and reagents are utilized. Potassium dihydrogen phosphate (KH2PO4) of HPLC grade is sourced from Qualigens and used specifically for these drugs. Similarly, formic acid of HPLC grade from Qualigens is employed for the estimation of Tamsulosin and Tadalafil. Water of HPLC grade from Qualigens is utilized for all drugs. Acetonitrile, another HPLC grade chemical from Qualigens, is also used for all drugs. Lastly, methanol of HPLC grade from Rankem is employed across all drugs.

Equipment's / instruments used:

For the analytical procedures, an electronic balance, model SAB2032, manufactured by Scaletech is utilized. An ultra-sonicator, model SE60US from Labman Scientific India, is employed for ultrasonic cleaning. Thermal treatments are conducted using a thermal oven, model i-THERM A17782, by Dwaraka Scientific. A pH meter, model ORION STAR A111, from Thermo Scientific is used for accurate pH measurements. Filtration tasks involve filter paper with a pore size of 0.45 microns, provided by Millipore. Lastly, the HPLC system in use is the Waters 2690 Separation Module, manufactured by Waters.

Method development:

Choosing λmax:

Spectrum of UV with 10µg/ml TamsulosinHcl &Taladafilin MP ratio) was noted by examining in the scale of 200 to 400nm and the isobestic λmax of both the drugs obtained at243 nm.

Optimization of Column:

DIKMASPURSILC18-EP(3.0x150mm,3µm))is find out optimum as it produce excellent shape of peak & RS at 1.0 ml per min flow speed.

Optimized Chromatographic Conditions

Instrument: RP-HPLC having Auto Sampler and PDA detector
Column : SPURSIL C18-EP (3.0x150mm,3µm)
MP ratio : 0.1%Formic acid: ACN (66:34ml)
Flow : 1ml per min
λmax : 243nm
Volume Injected : 10 µl
Run duration : 10min.

Buffer & mobile phase making:

0.1% Formic acid pH 4.5:
By adding 0.1ml of formic acid in a 1L water. Adjust this solution to pH 4.5 by using sodium hydroxide.
Mobile phase Making:
Mix a 660ml Formic acid (66%), 340 ml ACN (34%) & remove gases in ultra-sonication water bath for few min. Filter by vacuum filtration instrument using 0.45µ filter paper.
Diluent: 0.1% Formic acid ph 4.5: ACN (66%: 34%)

System Suitability:

Tailing factor for Tamsulosin &Taladafilin Std solution shouldn't>2.0.
For Standard solution Theoretical plates for the Tamsulosin & Taladafil peaks should n 't<2000.

Calculation: (For Tamsulosin &Taladafil)

$$\%\,Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Assay:

Standard Solution Preparation:

Precisely measure & poured 2mg of Tamsulosin& 25mg of Taladafil standard into a 20ml VF add Diluents & to dissolve fully sonicate& fill up till the margin. Additional take out 0.6ml from solutions into a 10ml VF & fill up till the margin using dilutant. (4.8 ppm Tamsulosin & 60ppm Taladafil).

Sample Solution Preparation:

Precisely measure & poured equal to 2mg of Tamsulosin& 25mg of Taladafil equal wt of the sample into a 10ml VF add few ml of dilutant & to fully dissolve sonicate fully get volume up till the margin. Additional take out 0.6ml from solutions into a 10ml VF & fill up till the margin using dilutant. (4.8ppm Tamsulosin & 60ppmTaladafil).

Procedure: Inject 10 µL from solutions into a 10ml VF & fill up till the margin using dilutant.

3. Results and Discussion

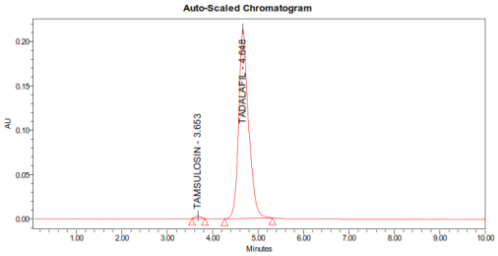

Figure 3: Optimized Chromatogram

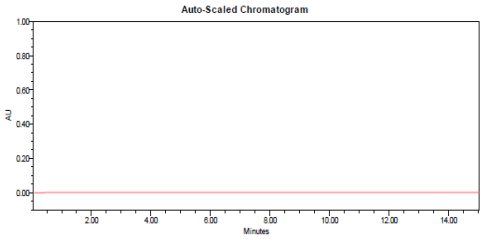

Figure 4: Blank Chromatogram

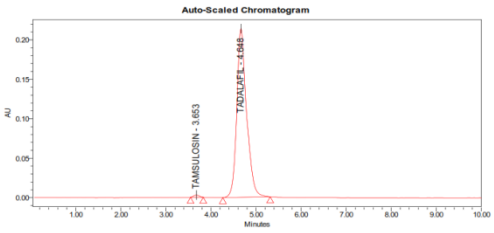

Figure 5: Standard Chromatogram

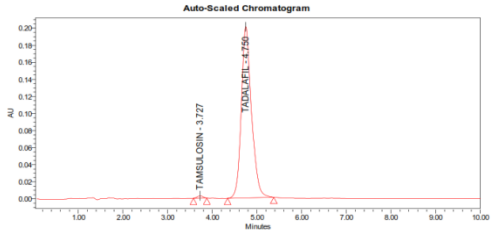

Figure 6: Sample Chromatogram

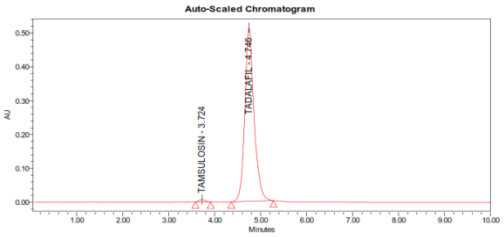

Figure 7: Linearity Chromatogram

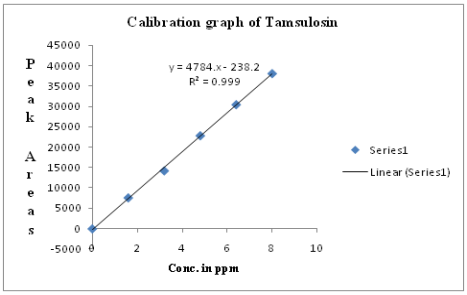

Figure 8: Tamsulosin Calibration graph

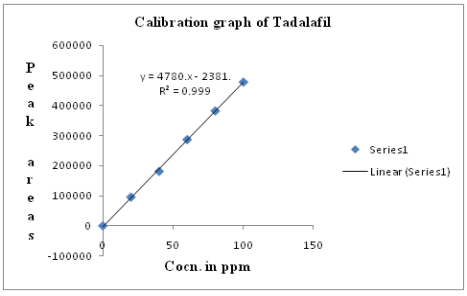

Figure 9: Tadalafil Calibration graph

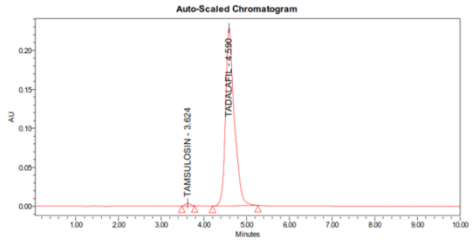

Figure 10: Precision Chromatogram

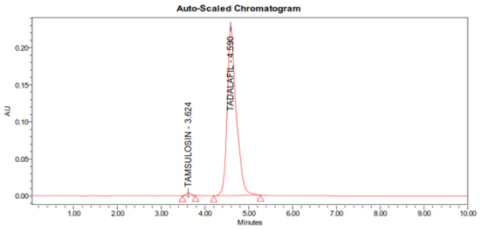

Figure 11: ID Precision Chromatogram

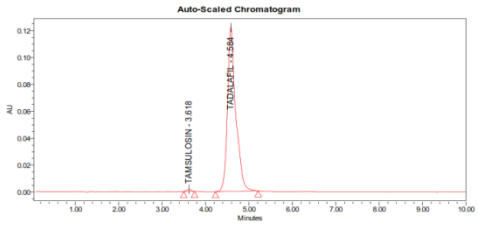

Figure 12: Accuracy 50%-3 Chromatogram

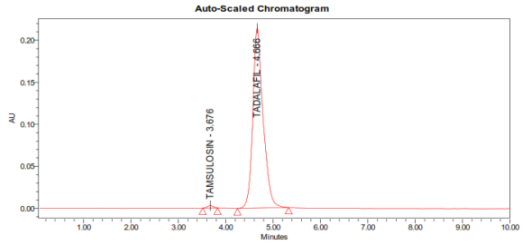

Figure 13: Accuracy 100%-3 Chromatogram Chromatogram

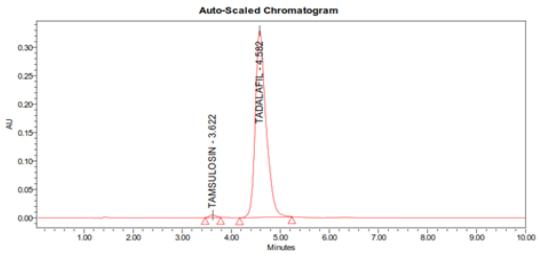

Figure 14: Accuracy 150%-3 Chromatogram

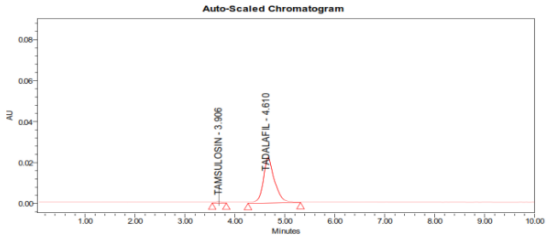

Figure 15: Tamsulosin &Tadalafil depicting LOD Chromatogram

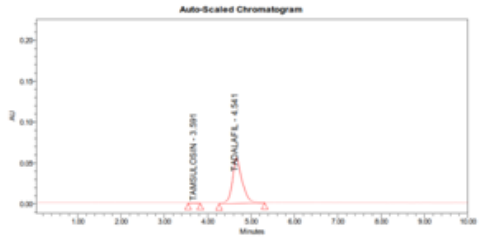

Figure 16: Tamsulosin and Tadalafil depicting LOQ Chromatogram

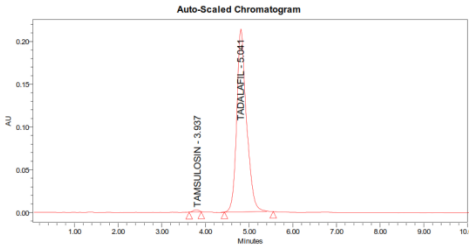

Figure 17: less flow Chromatogram

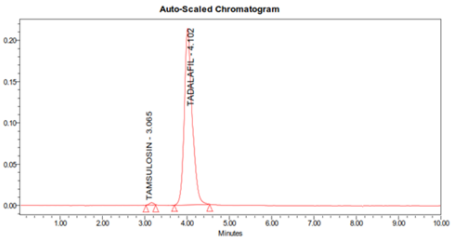

Figure 18: more flow Chromatogram

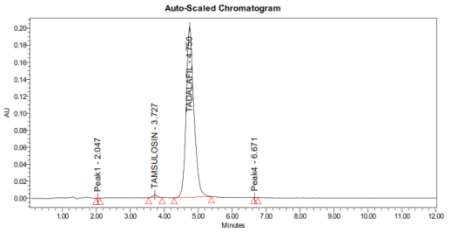

Figure 19: Acid Degradation Chromatogram

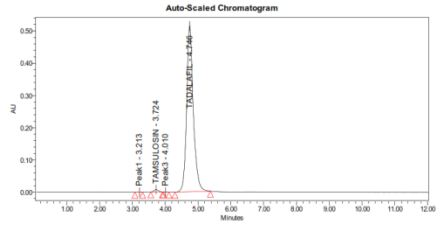

Figure 20: Base Degradation Chromatogram

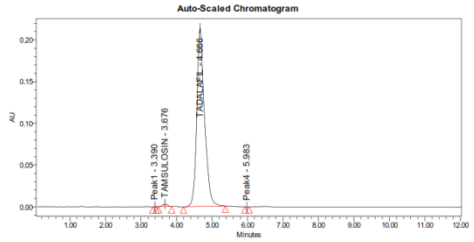

Figure 21: Peroxide Degradation Chromatogram

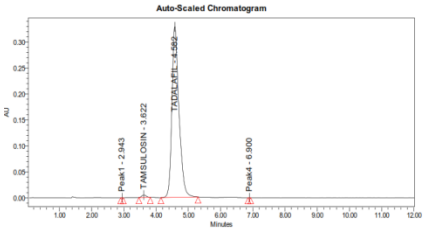

Figure 22: Thermal Degradation Chromatogram

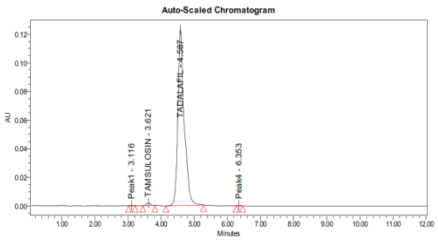

Figure 23: Photo Degradation Chromatogram

Table 1: LOD Results

Drug name	noise of Baseline(µV)	Obtained Signal O(µV)	Signal /Noise ratio	Conc. In ppm
Tamsulosin	80	171	2.975	0.026µg/ml
Tadalafil	80	238	2.9	0.027 µg/ml

Table 2: LOQ Results

Drug name	noise of Baseline(µV)	obtained Signal (µV)	Signal /Noise ratio	Conc. In ppm
Tamsulosin	80	788	9.85	0.092µg/ml
Tadalafil	80	798	9.9	0.093µg/ml

Table 3: Outcomes of difference in flow speeds for Tamsulosin&Tadalafil

S. No	Flow Rate(ml per min)	SST outcomes of tamsulosin	
		Plate Count	Tailing
1	0.8	2422	1.2
2	1.0	2426	1.0
3	1.2	2439	1.5

S. No	Flow Rate(ml per min)	SST outcomes of tadalafil	
		Plate Count	Tailing
1	0.8	6832	1.01
2	1.0	6838	1.07
3	1.2	6844	1.08

Table 4: Outcomes for difference in MP Ratios for Tamsulosin and Tadalafil

S.No	Differin Organic Composition in the MP	SST outcomes of tamsulosin	
		USP Plate Count	USP Tailing
1	10% less	2422	1.2
2	*Actual	2426	1.0
3	10% more	2439	1.5

S. No	Differin Organic Composition in the MP	SST outcomes of tadalafil	
		Plate Count	Tailing
1	10% less	6832	1.01
2	*Actual	6838	1.07
3	10% more	6844	1.08

Table 5: Degradation outcomes for Tamsulosin&Tadalafil

Parameters	Tamsulosin	
	Areas	% Degraded
Standard	22878	
Acid	20997	8.22
Base	21436	6.30
Peroxide	20953	8.41
Thermal	20847	8.88
Photo	20934	8.50

Parameters	Tadalafil	
	Areas	% Degraded
Standard	285972	
Acid	284974	0.35
Base	284361	0.56
Peroxide	279539	2.25
Thermal	265479	7.17
Photo	263432	7.88

4. Conclusion

A stability-indicating RP-HPLC technique was setup& validated for the parallel determination of Tamsulosin HCl &Tadalafil in dosage forms. Tamsulosin HCl is used to treat benign prostatic hyperplasia, while Tadalafil is prescribed for erectile dysfunction. The primary goal of this study was to develop a precise, accurate, & robust technique capable of simultaneously estimating both drugs, as well as detecting any degradation products formed under stress conditions (e.g., acid, base, oxidation, heat, and light) for stability testing. The constructed stability-indicating RP-HPLC technique was found to be precise, accurate, &robust for the parallel identification of Tamsulosin HCl &Tadalafil in dosage forms. The technique demonstrated its ability to separate the drugs from their potential degradation products under various stress conditions, making it suitable for stability testing. This validated technique complies with ICH rules &can be routinely used in QC laboratories for the analysis of Tamsulosin HCl &Tadalafil in combined formulations, ensuring product stability & efficacy.

5. References

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