RESEARCH ARTICLE

RP-HPLC-Based Stability-Indicating Analytical Method for Concurrent Determination of Finasteride and Tadalafil: Development and Validation

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ABSTRACT
A stability-indicating RP-HPLC method was developed for the simultaneous estimation of Finasteride and Tadalafil using a Symmetry C18 column and an Acetonitrile:0.1% TEA buffer (pH 2.5, 20:80) mobile phase. Detection at 257 nm, flow rate of 1.0 mL/min, and 6-minute runtime ensured effective separation. The method showed linearity from 12.5–75 µg/mL with $R^2 > 0.999$, precision with %RSD < 2%, and LOD/LOQ values of 0.60 µg/mL and 2.00 µg/mL. Assay results for the marketed formulation (ENTADFI) showed 100.0% and 100.2% for Finasteride and Tadalafil, respectively. Forced degradation studies confirmed that Finasteride and Tadalafil were susceptible to degradation under acid, alkali, and oxidative conditions, with maximum degradation observed under acidic (14.0%) and oxidative (14.7%) stress for Finasteride, and under alkali (10.8%) and reduction (14.6%) for Tadalafil. Minimal degradation was seen under thermal and hydrolytic conditions

Keywords: Finasteride, Tadalafil, RP-HPLC, Method, Validation, ICH, Forced degradation
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INTRODUCTION

Finasteride (Fig.1) a 5α-reductase inhibitor, is utilized in the treatment of androgen-dependent conditions such as benign prostatic hyperplasia and alopecia [1,2]. Its mechanism involves the suppression of DHT synthesis, which alleviates symptoms associated with excess androgen activity. Tadalafil acts by inhibiting PDE-5, thereby maintaining cGMP levels that lead to vasodilation and are beneficial in treating erectile dysfunction and pulmonary hypertension [3,4]. The combination of these agents, as approved in Entadfi™, addresses overlapping urological conditions with improved patient compliance and therapeutic synergy [5,6]. Multiple methods have been reported for the combined analysis of Finasteride and Tadalafil, including UV, RP-HPLC, LC-MS/MS, and green spectrophotometric techniques [7-11]. Our study focuses on a validated RP-HPLC method designed to be stability-indicating under ICH-recommended stress conditions.

MATERIALS AND METHODS

Chemicals and Reagents

Finasteride and Tadalafil were obtained from Yarrow Chem Products. Acetonitrile and Methanol (HPLC-grade) were from Merck, while Triethylamine and Orthophosphoric acid (AR grade) were sourced from SD Fine-Chem Ltd.

Instruments

A Waters Alliance HPLC system (e2695 model), operated via Empower 2.0 software, was utilized for performing the analysis. A Eutech pH700 pH meter and a Sartorius BSA224S-CW analytical balance were used. Class-A glassware (Borosil), a Unichrome UCA 701 Ultrasonicator, and a Waters Isocratic pump (INN model) supported the experimental setup.

Preparation of Stock and Sample Solution

Stock solution was prepared by dissolving 5 mg of Finasteride and Tadalafil in diluent, sonicated, and diluted to 10 mL. A 1 mL portion was diluted to 10 mL to yield 50 µg/mL. Similarly, 88 mg of the sample was processed, diluted, and filtered to produce an equivalent 50 µg/mL sample solution.

Method Development

A series of chromatographic trials (Table 1) were conducted using various mobile phases and columns to optimize the RP-HPLC method for Finasteride and Tadalafil. Among all, Trial-6 with a Symmetry C18 column and ACN + 0.1% TEA buffer (20:80, pH 2.5) yielded sharp, well-resolved peaks with acceptable system suitability parameters and was selected as the optimized method for validation.

Method Validation

The method validation tests, such as selectivity, sensitivity, range, specificity, linearity, and accuracy, were studied to evaluate the method's validity. The approach was also tested for Finasteride and Tadalafil in bulk and pharmaceutical form. Each validation parameter was assessed in strict compliance with ICH guidelines [12-15].

System Suitability

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Table 1: Optimization Trials of Chromatographic Conditions

Trial No.	Column	Mobile Phase	Wavelength (nm)	Observation
Trial-1	Agilent Eclipse XDB (250×4.6 mm, 5 µm)	Acetonitrile + 0.1% TFA (70:30)	200–400	Retention time is not within the limit
Trial-2		Acetonitrile + 0.1% TFA (60:40)	257	Baseline is not sufficient
Trial-3		Acetonitrile + 0.1% TFA (50:50)		Plate count and tailing are not within the limit
Trial-4	Symmetry C18 (150×4.6 mm, 3.5 µm)	ACN + 0.1% TEA pH 2.5 / OPA (10:90)		Broad peak is observed
Trial-5		ACN + 0.1% TEA pH 2.5 / OPA (20:80)		Response of the peaks is very high
Trial-6		ACN + 0.1% TEA pH 2.5 / OPA (20:80)		This method is suitable for validation

To ensure consistent system performance, a system suitability test was performed in accordance with ICH Q2(R1). Standard solutions of Finasteride and Tadalafil were injected six times, and parameters including retention time, theoretical plates, tailing factor, and %RSD were assessed. All values fell within acceptable limits, validating the system's readiness for routine analysis

Table 2: HPLC System Suitability Results for Finasteride & Tadalafil

Parameter	Finasteride	Tadalafil
Retention time	2.018	3.651
Plate count	13546	12980
Tailing factor	1.09	0.99
Resolution	----	9.58
%RSD	0.32	0.68

Specificity

The specificity of identification tests, impurity determinations, and assays should be investigated during their validation. To demonstrate specificity, representative chromatograms need to be utilized, and extracted components must be labeled consistently. An appropriate level of investigation must be applied to critical separations in chromatography. The components' resolution that elutes nearest to each other could be employed to assess the specificity of a significant separation. Analytical procedures with higher levels of specificity may compensate for less specific individual procedures.

Linearity, Precision

Linearity is recommended to evaluate the method such that the obtained results produce a proportional response over the desired range of analyte concentrations. Linearity was

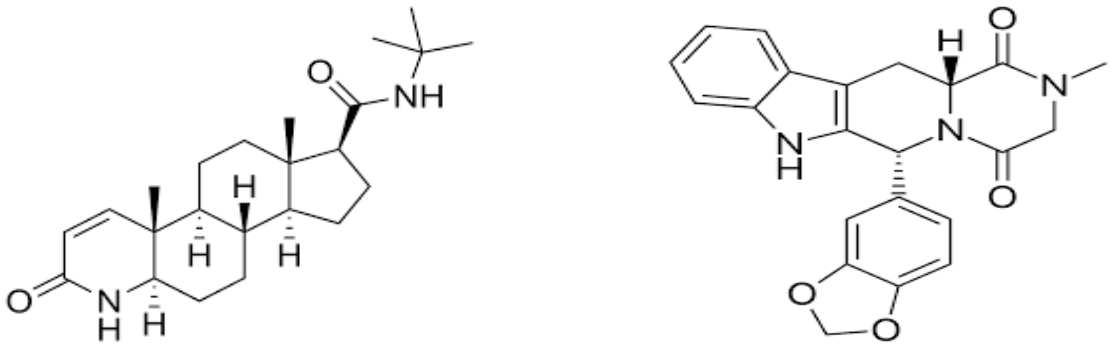

Figure 1: Structure of Finasteride and Tadalafil

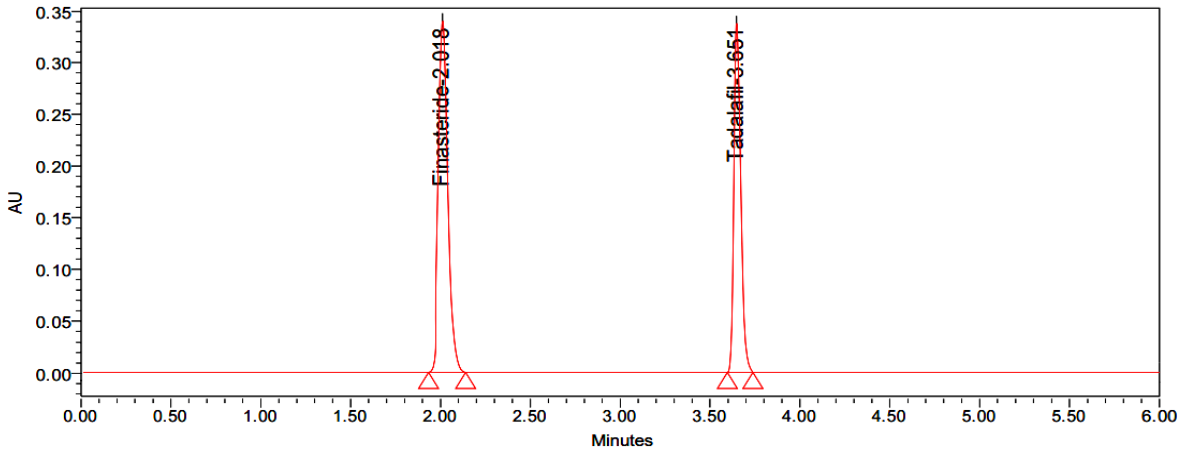

Figure 2: Optimized chromatogram during Method Development

Table 3: Results of linearity for Finasteride & Tadalafil

S.No.	Finasteride		Tadalafil	
	Std. Conc. (µg/ml)	Area	Std. Conc. (µg/ml)	Peak Area
1	12.50	968872	12.50	938374
2	25.00	1937745	25.00	1876748
3	37.50	2906617	37.50	2815122
4	50.00	3875491	50.00	3753496
5	62.50	4814363	62.50	4691870
6	75.00	5753235	75.00	5530244
Regression equation	y = 76824.09x + 12856.89		y =74212.78x + 17857.14	
Slope	76824.09		74212.78	
Intercept	12856.89		17857.14	
R^2	0.99998		0.99989	

Table 4: System and Method precision of Finasteride and Tadalafil

	Concentration Finasteride and Tadalafil (50µg/ml)			
S.No.	System-Precision		Method -Precision	
1.	FNS Area	TDL Area	FNS Area	TDL Area
2.	3879791	3752957	3846491	3756921
3.	3854689	3724889	3897979	3785787
4.	3869915	3795454	3857801	3772140
5.	3877998	3762210	3887757	3766124
6.	3849154	3782799	3891504	3750491
Mean	3862105	3746430	3881136	3762174
S.D	3865609	3760790	3877111	3765606
%RSD	12453.510	25473.330	20408.970	12376.634
	0.32	0.68	0.53	0.33

determined by regression analysis, which involves recording average areas of triplicate injections of 12.50-75.00. µg/mL for Finasteride and Tadalafil. Plot a linearity graph with concentration against peak area, and for Finasteride and Tadalafil correlation coefficient values were acceptable fits for the data of the regression line. All the drugs exhibit a proportional response with a good coefficient of correlation of 0.999.

Repeatability is to generate the same results over a short time interval under identical conditions. Intermediate precision is the precision attained over an extended period in a single laboratory but with different analysts, different columns, different reagents, etc.

Accuracy

Samples were arranged in triplicate by sample solution at known concentrations of sodium Finasteride and Tadalafil at 50%, 100%, and 150% spike levels. The peak area values observed in each analysis were compared with the corresponding standard and the % recovery of Finasteride and Tadalafil was calculated. The % recovery in the limit of 98-102 % was considered as acceptable as per the guidelines.

Robustness

It is evaluated to know the change in system suitability parameters in response to variations in flow rate, mobile phase, and temperature. % RSD was calculated for evaluation of the parameters and found to be less than 2%, which satisfies the acceptance criteria.

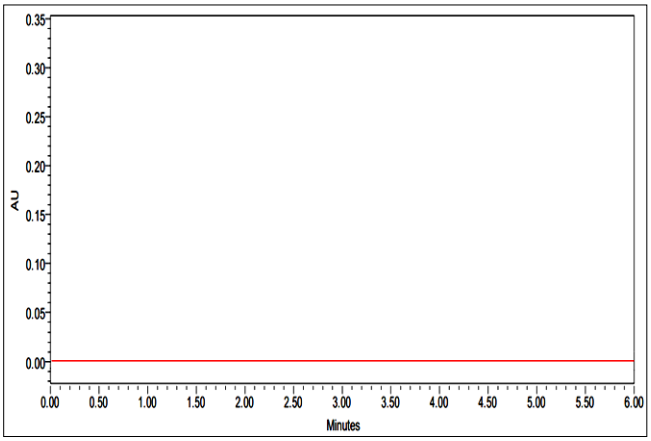

Figure 3: Chromatogram of blank

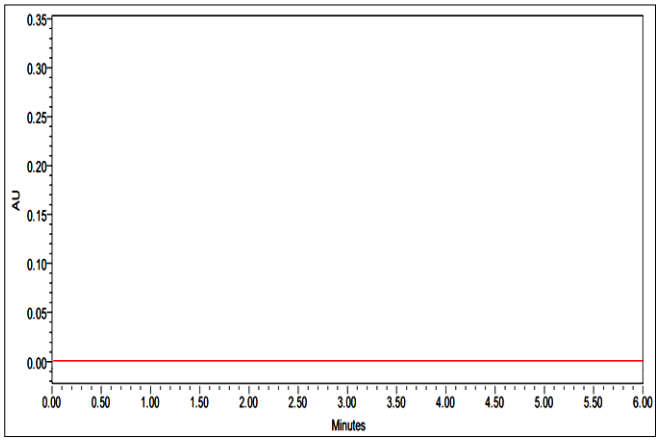

Figure 4: Chromatogram of placebo

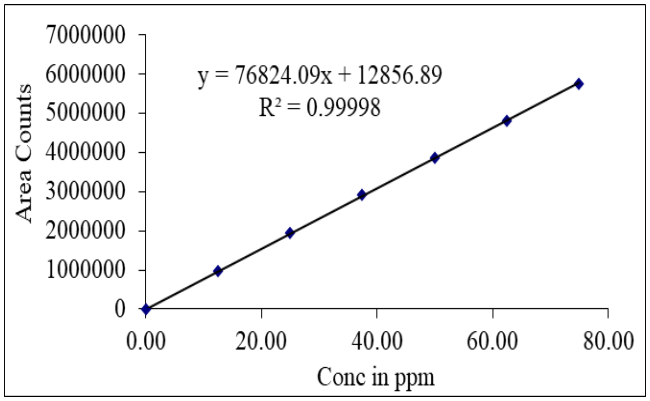

Figure 5: Linearity Plot for Finasteride

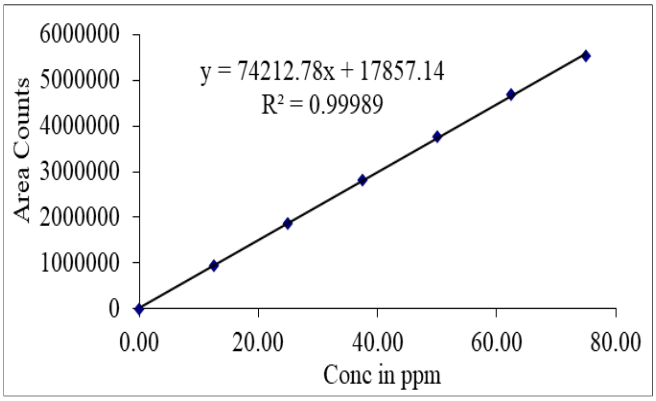

Figure 6: Linearity Plot for Tadalafil

Assay

Using 10 µL injections, standard and sample solutions were analyzed via HPLC. Peak responses were measured, and the assay of Finasteride and Tadalafil was calculated by comparing to the standard.

Degradation Studies

Acid and alkali degradation involved treatment with 1N HCl or NaOH, followed by neutralization and dilution. Thermal degradation was done by heating at 105°C for 6 hours. Oxidation and reduction were carried out using 10%

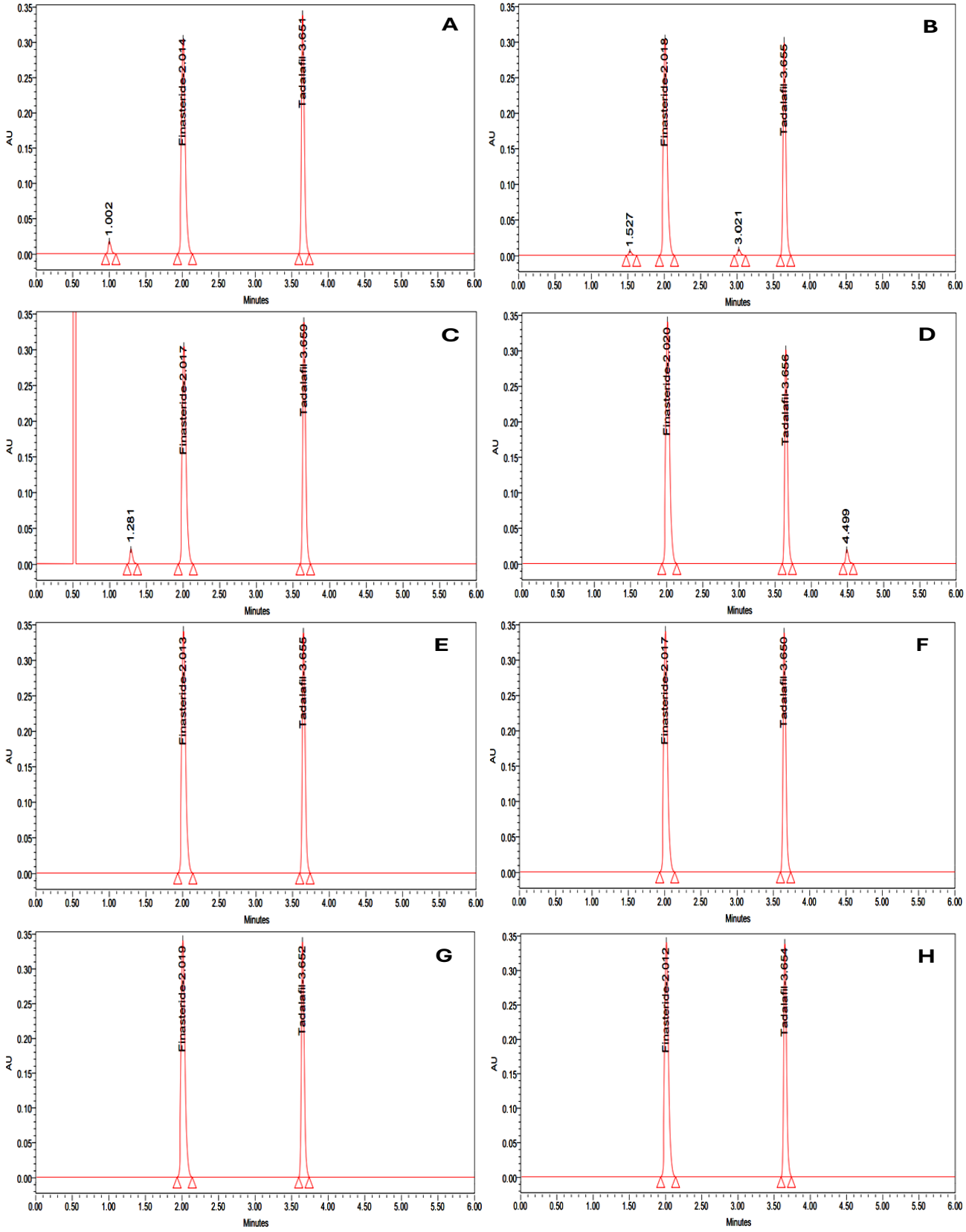

Figure 7: Degradation chromatograms of Acid, Alkali, Peroxide, Reduction, Thermal, Photolytic, Hydrolysis and Control

Table 5: Recovery Studies of Finasteride and Tadalafil

Accuracy	Finasteride		Tadalafil	
%Conc Level	% Recovery	Mean Recovery	% Recovery	Mean Recovery
50%	100.0	100.7	100.2	100.3
	101.2		99.9	
	100.8		100.8	
100%	99.8	100.3	100.0	100.2
	100.6		100.7	
	100.4		99.9	
150%	100.3	100.6	100.2	100.0
	100.5		99.8	
	100.9		100.1	

hydrogen peroxide and 10% sodium bisulfite. Photolytic stress was applied using 1.2 million lux and 200 Wh/m² UV, while hydrolysis was conducted with water for 30 minutes at room temperature [16-19].

RESULTS AND DISCUSSIONS

Method Optimization

Using a Waters HPLC with PDA detector (Fig. 2), optimal separation of Finasteride and Tadalafil was achieved on a Symmetry C18 column with a 20:80 Acetonitrile:0.1% TEA buffer (pH 2.5) mobile phase. The method utilized a 1 mL/min flow, 257 nm detection, and 6-minute runtime.

Method Validation

System Suitability

As presented in Table 2, System suitability parameters for Finasteride and Tadalafil met the USP acceptance criteria, showing sharp peaks with good resolution (9.58 for Tadalafil), acceptable tailing factors, and low %RSD values.

Specificity

To establish specificity, blank samples were analyzed and found to be free from any peaks at the retention times of Finasteride and Tadalafil, as demonstrated in Figures 3 and 4.

Linearity, LOD & LOQ

Both Finasteride and Tadalafil exhibited strong linear responses ($R^2 > 0.9998$) over the 12.5–75 µg/mL range. Details are presented in Table 3 and Figures 5–6. The LOD and LOQ values were 0.60 µg/mL and 2.00 µg/mL for each drug.

System Precision and Method Precision

As presented in Table 4, the %RSD values for both system and method precision were found to be well below the acceptable limit of 2%, indicating excellent repeatability. Finasteride showed %RSD values of 0.32% (system) and 0.53% (method), while Tadalafil demonstrated 0.68% and 0.33%, respectively.

Accuracy

As shown in Table 5, the percentage recovery for Finasteride ranged from 99.8% to 101.2%, with mean recoveries of 100.7%, 100.3%, and 100.6% for the 50%, 100%, and 150% levels, respectively. Similarly, Tadalafil exhibited recovery values between 99.8% and 100.8%, with mean recoveries of 100.3%, 100.2%, and 100.0% across the corresponding levels.

Assay

The % assay values were found to be 100.0% for Finasteride and 100.2% for Tadalafil.The assay was performed using standard and sample concentrations of 50 µg/mL for both drugs, and the amount found closely matched the label claim of 5 mg per tablet, as detailed in Table 6.

Table 6: Assay of Finasteride & Tadalafil

Brand	Drug	Avg sample area (n=2)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Amount found (mg)	% assay
ENTADFI	Finasteride	3862699	50	50	5.00	100.0
	Tadalafil	3768533	50	50	5.01	100.2

Table 7: Robustness Results of Finasteride and Tadalafil

	Condition	Finasteride			Tadalafil		
		Rt(min)	Area	% CV	Rt(min)	Area	%CV
Flow rate (mL/min)	Less flow (0.9ml)	2.237	3657471	0.21	3.775	3570949	0.20
	Actual (1.0ml)	2.018	3879791	0.32	3.651	3752957	0.68
	More flow (1.1ml)	1.946	4155459	0.31	3.465	3868797	0.26
Organic Phase change	Less Org (18:82)	2.401	3491547	0.49	3.957	3349724	0.49
	Actual (20:80)	2.016	3854689	0.32	3.653	3724889	0.68
	More Org (22:78)	1.825	4277491	0.15	3.306	4054811	0.23

Table 8: Forced Degradation results for Finasteride and Tadalafil

Degradation conditions	Finasteride				Tadalafil			
	Assay %	Degradant %,	PA	PT	Assay %	Degradant %	PA	PT
Control	100	0	3.456	8.526	100	0	6.958	13.524
Acid	86.0	14.0	3.454	8.571	98.8	1.2	6.988	13.541
Alkali	90.1	9.9	3.463	8.576	89.2	10.8	6.932	13.586
Peroxide	85.3	14.7	3.458	8.587	98.1	1.9	6.941	13.552
Reduction	97.5	2.5	3.403	8.524	85.4	14.6	6.984	13.579
Photolytic	98.9	1.1	3.442	8.553	96.2	3.8	6.953	13.501
Hydrolysis	98.0	2.0	3.431	8.557	99.5	0.5	6.911	13.514
Thermal	99.7	0.3	3.429	8.539	98.3	1.7	6.905	13.579

Robustness

As shown in Table 7, the robustness study was conducted by varying flow rate and organic phase composition. A decrease in flow rate slightly increased the retention times (RT) for both Finasteride and Tadalafil, while higher flow rates resulted in shorter RTs and increased peak areas. Similarly, altering the organic phase showed that increasing the organic content decreased RT and enhanced peak response. Across all conditions, %RSD values remained below 1%, indicating the method's precision and robustness against small deliberate changes in chromatographic parameters.

Degradation Studies

Forced degradation studies confirmed that Finasteride and Tadalafil were susceptible to degradation under acid, alkali, and oxidative conditions, with maximum degradation observed under acidic (14.0%) and oxidative (14.7%) stress for Finasteride, and under alkali (10.8%) and reduction (14.6%) for Tadalafil. Minimal degradation was seen under thermal and hydrolytic conditions. Peak purity parameters (purity angle < purity threshold) indicated no co-eluting degradants, confirming the method's specificity. These results, along with degradation percentages, are summarized in Table 16 and depicted in Figure 7.

CONCLUSION

A precise and validated RP-HPLC method was developed for the simultaneous estimation of Finasteride and Tadalafil, suitable for bulk and dosage form analysis. The optimized chromatographic conditions provided sharp, well-resolved peaks with acceptable system suitability parameters. Forced degradation studies under various stress conditions confirmed the method’s ability to specifically separate the active drugs from their degradation products, highlighting its stability-indicating capability. The method also proved reliable in quantifying the drugs in the marketed formulation ENTADFI, with assay results close to 100% for both Finasteride and Tadalafil. Overall, this RP-HPLC method offers a simple, reproducible, and efficient analytical approach for the simultaneous estimation and stability assessment of Finasteride–Tadalafil combination products, making it suitable for application in pharmaceutical industry and regulatory laboratories.

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