NOVEL STRESS INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FINASTERIDE AND TADALAFIL

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Abstract:*A novel stability indicating RP-HPLC method was developed for the simultaneous estimation of Finasteride (FSD) and Tadalafil (TDF) in pharmaceutical dosage form. Effective chromatographic separation achieved using a Waters Luna Phenyl Hexyl C18 column (250 mm x 4.6 internal diameter, 5µ particle size) with mobile phase Acetonitrile: Ammonium formate pH-3.0 with OPA (70:30 % v/v) at a flow rate of 1 ml/min. The analytes were monitored at 228 nm using a photo diode array detector at ambient temperature. The retention times for FSD and TDF were about 2.415 and 7.263 mins, respectively. Calibration curves were linear in the ranges of 12.5 to 75 µg/ml for FSD and 12.5 to 75 µg/ml for TDF with correlation coefficients > 0.99. The method has the requisite accuracy, selectivity, sensitivity, precision and robustness .Degradation products obtained from the stress studies did not interfere with the detection of FSD and TDF. The validated RP-HPLC method was effectively used to the analysis of FSD and TDF in marketed dosage form.*

Key words: *Finasteride, Tadalafil, RP-HPLC, Stability indicating*

INTRODUCTION

Finasteride (FSD), Finasteride, also known as 17β-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one, is a synthetic androstane steroid and 4-azasteroid. It is an analogue of androgen steroid hormones like testosterone and DHT. **Fig. 1a**, is a specific inhibitor of steroid Type II 5α-reductase, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone. It is commonly used to treat benign prostatic hyperplasia, prostate cancer, and androgenetic alopecia..

Tadalafil (TDF), chemically (6R,12aR)-6-(1,3-Benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione, **Fig.1b** is a potent and selective phosphodiesterase-5 (PDE-5) inhibitor, a secondary messenger for the smooth muscle-relaxing effects of nitric oxide, which plays an important role in the vasodilation of erectile tissues.

There are different methods like UV, Liquid chromatography-tandem mass spectrometric (LC–MS/MS) methods were developed for the simultaneous determination of FSD and TDF.LC–MS/MS is a specific instrumental based technique affects the cost and the speed of the quantitative analysis of the compounds of interest. Therefore, authors are made an attempt to develop the new stability indicating RP-HPC method for simultaneous determination of FSD and TDF in pure and pharmaceutical formulation.

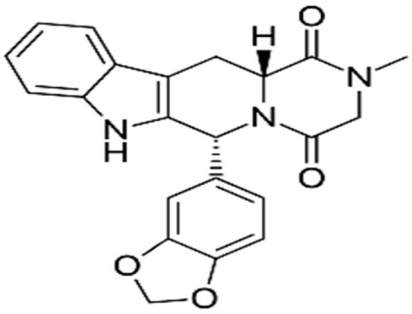

Figure: 1a Finasteride

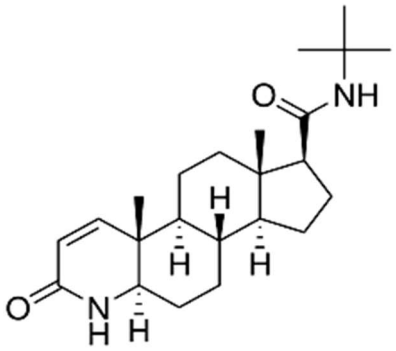

Figure 1b: Tadalafil

EXPERIMENTAL

Materials and Methods

FSD was procured from Dr Reddy's Laboratories and TDF was supplied by Rakshith Pharmaceuticals, Hyderabad as gift samples. All the reagents used were of HPLC grade.

Equipment

Analysis was carried out by using Waters Alliance HPLC 2695 System fitted with quaternary pumps, Photo Diode Array Detector, and Auto Sampler integrated with Empower 2 software.

Chromatographic Conditions

Mobile phase consisting of Acetonitrile: Ammonium formate pH-3.0 with OPA (70:30 % v/v) and it was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 10µL. PDA detection was performed at 228 nm and the separation was achieved at ambient temperature.

Pharmaceutical formulations

A commercial product (Entadfi™ labeled contains 5 mg FSD and 5 mg TDF per capsule) was studied

Preparation of standard stock solution

Accurately weighed quantities (5mg each) of FSD and TDF were dissolved separately in sufficient quantity of mobile phase in a 10mL volumetric flask. The volume was adjusted up to the mark with mobile phase to obtain a stock solution of mg/mL each of FSD and TDF.

RESULTS AND DISCUSSION

Method Development

Several HPLC - UV analytical methods were published for the estimation of FSD and TDF alone or in combination with other drugs in bulk and pharmaceutical dosage forms and there were no methods reported on FSD and TDF combination. Also the published methods were not economical. The aim of the present work was to develop and validate a simple, efficient, sensitive and selective method for the simultaneous estimation of FSD and TDF in bulk and dosage form. In the present investigation, initial trials were made to develop LC conditions for the separation of FSD and TDF using Acetonitrile and 0.1% OPA (80:20 v/v) at a flow rate of 1.0 mL/min, With X-Bridge Phenyl (150x4.6 mm,3.5µ) at a flow rate of 1.0 mL/min and the Peaks are not separated properly. In another trial using Acetonitrile : Ammonium formate pH-3.0 with OPA (60:40 v/v) at a flow rate 1.0 ml/min, with Luna Phenyl Hexyl (250x4.6 mm, 5µ) column Plate count is not within the limit. Whereas, with the same column with change in mobile phase to Acetonitrile: Ammonium formate pH-3.0 with OPA (65:35 v/v) at a flow rate of 1.0 mL/min FSD was eluted at 2.291 min and TDF was at 8.719 min with broad peak shape. Then by changing mobile phase composition of (70:30 v/v), a good resolution with sharp peaks were obtained (**Figure 2**), and these conditions were finalized for simultaneous estimation of FSD and TDF in bulk and pharmaceutical dosage forms. For quantitative analytical purpose wavelength was set at 228 nm, which provided better reproducibility with minimum or no interference.

Method validation

The method described above has been validated as per the ICH guidelines (ICH–Guidelines Q2B, Switzerland, 1996) and the results were summarized below.

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose 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**.**

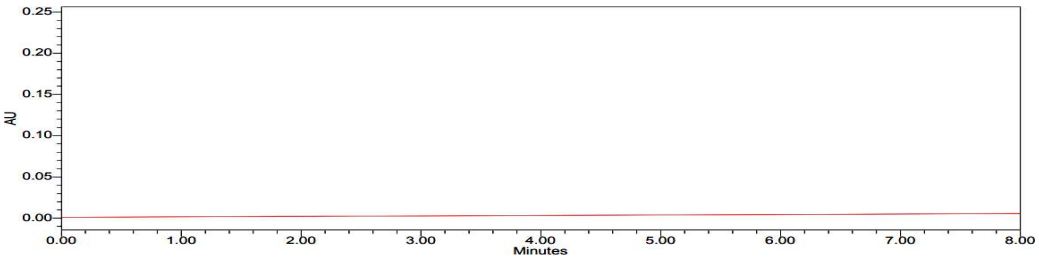

Figure 2 : Chromatogram of blank

System suitability

System suitability was carried out by injecting 50 µg/mL of FSD and TDF at different injection volumes in the range of 12.5 µg/mL to 75µg/mL. With increment of injection volumes, the %RSD for tailing factor and theoretical plate number was less than 1% and is satisfactory and data is given in **Table 1**.

Table 1: System suitability parameters for Finasteride & Tadalafil

S.No	Parameter	Finasteride	Tadalafil	Accepted limit
1	Capacity factor(K)	3.67	2.64	>2
2	Plate count(N)	5349	5876	>2000
3	Tailing factor(Tf)	1.15	1.04	< 2.0
4	Resolution(Rs)	----	21.50	>2.0
5	%RSD	0.52	0.48	< 2.0%

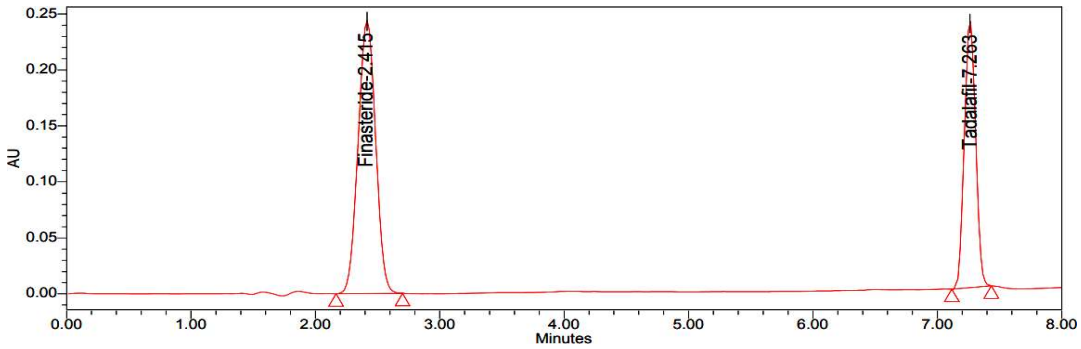

Figure 3 : Chromatogram of standard

Linearity

A linear relationship was evaluated across the range (12.5-75 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 50-150 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient (R^2) for both the drugs were found to be > 0.99 and indicating good linearity. The linearity data is given in **Table 2** and **Figure 2&3.**

Table 2: Linearity of Finasteride and Tadalafil

S.No	Finasteride		Tadalafil	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	12.50	588487	12.50	618837
2	25.00	1161250	25.00	1222790
3	37.50	1751664	37.50	1718985
4	50.00	2330781	50.00	2361081

5	62.50	2863848	62.50	2952775
6	75.00	3519293	75.00	3504261
Regressin equation	y = 46494 x +1793		y = 46307 x + 37204	
Slope	46494		46307	
Intercept	1793		37204	
R^2	0.999		0.999	

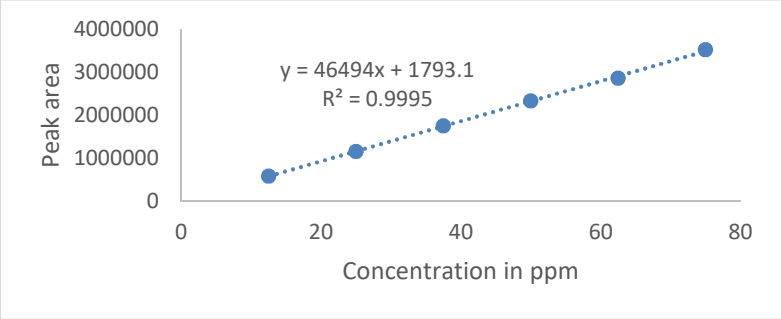

Fig 2: Linearity curve of Finasteride

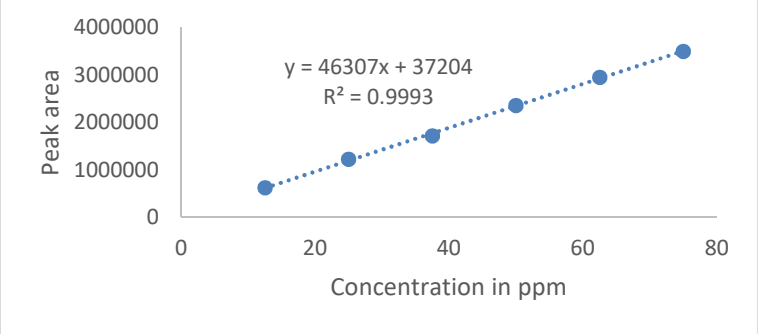

Fig 3 : Linearity curve of Tadalafil

Precision

Precision studies were carried out in terms of repeatability and reproducibility. Six replicate determinations were carried out and percent relative standard deviation for both the drugs was less than 2%, indicating the high degree of precision and results were given in **Table 3**.

Table 3: Method Precision for Finasteride & Tadalafil

S. No.	Area for Finasteride	Area for Tadalafil
1	2359715	2341128
2	2380789	2352087
3	2369223	2344278
4	2345136	2365687
5	2389054	2311713
6	2351732	2332174
Average	2365942	2341178
Standard Deviation	16964.39	18322.49
%RSD	0.72	0.78

Accuracy (Recovery)

Accuracy was determined by the recovery study of known amounts of FSD and TDF standards added to a sample solution. Different concentrations of the two active ingredients were added to the sample and the recovery was measured. The data obtained for the evaluation of linearity were used. The accuracy as reflected from the recovery data and statistical evaluation of the assay for

the two active ingredients is listed in **Table 4&5**.

Table 4:Accuracy results of Finasteride

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1183115	2.5	2.5	100.0	99.57
100%	2355687	5	4.98	99.6	
150%	3515124	7.5	7.43	99.1	

Table 5:The Accuracy results for Tadalafil

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1180354	2.5	2.53	101.2	100.1
100%	2328120	5	5	100.0	
150%	3468324	7.5	7.44	99.2	

LOD and LOQ

LOD and LOQ were calculated from the average slope and standard deviation of y-intercepts of the calibration curve. LOD was found to be 0.143 µg/mL and 0.157 µg/mL respectively for FSD and TDF, LOQ was found to be 0.472 µg/mL and 0.534 µg/mL respectively for FSD and TDF and data is given in **Table 6**.

Table 6: **Sensitivity parameters (LOD & LOQ)**

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Finasteride	0.143	0.157

Tadalafil	0.472	0.534

Robustness

Robustness of the analytical method is the indication of its reliability. The method has the capacity to remain unaffected with deliberate changes as there was no signicant change in the peak area with the change in flow rate and organic phase of mobile phase. There was slight change in retention time with the change in flow rate and the other chromatographic parameters were not altered. The data is given in **Table 7&8**.

Table7:Robustness results of Finasteride by RP-HPLC

Parameter	**Finasteride**					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.8ml)	2.684	2553564		1.19	5444
	Actual (1ml)	2.415	2359707		1.17	5342
	More flow (1.2ml)	2.290	2194138		1.12	5279
Organic Phase change	Less Org (63:37)	2.700	2756501		1.16	5470
	Actual (70:30)	2.418	2380724		1.15	5355
	More Org (77:23)	2.225	1965248		1.08	5247

Table 8: Robustness results of Tadalafil by RP-HPLC

Parameter	Tadalafil					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.8ml)	7.685	2544562	23.01	1.13	5972
	Actual (1ml)	7.263	2325171	21.53	1.09	5869
	More flow (1.2ml)	6.958	2073124	22.68	1.06	5808
Organic Phase change	Less Org (63:37)	7.711	2627541	23.18	1.14	5942
	Actual (70:30)	7.269	2339721	21.50	1.10	5862
	More Org (77:23)	6.606	1822874	21.30	1.04	5776

Assay

Assay of FSD and TDF in Entadfi capsules was performed by the proposed method and the % assay of the both drugs were calculated as an average of 3 determinations, results were given in Table 10 . These results indicate that the present HPLC method can be successfully used for the simultaneous assay of FSD and TDF in bulk and dosage forms. The data is tabulated in **Table 9**

Table 9: Results of the market product

Finasteride Label claim (mg)	**Amount found(mg) ± SD (n=3)**	**% Assay ± SD (n=3)**	**Tadalafil Label claim (mg)**	**Amount found(mg) ± SD (n=3)**	**% Assay ± SD (n=3)**

5	4.99 ± 0.08	99.85 ± 1.28	5	4.97± 0.14	99.42 ± 0.65
5	4.97 ± 0.05	98.64 ± 0.73	5	5.03 ± 0.27	100.2 ± 0.27
5	5.04 ± 0.10	100.82± 1.16	5	4.96 ± 0.35	99.24 ± 1.25

Forced Degradation Studies

The ability of the proposed method to separate FSD and TDF from their degradation products was evaluated by intentional degradation via various stress conditions such as acid hydrolysis (using 1N HCl), base hydrolysis (using 1N NaOH), oxidation (using 3% V/V H2 O2) at 60°C, thermal degradation at 110°C and data was given in **Table 10** and Shown in Figures **6,7,8,9 & 10.**

Preparation of stock solution

Accurately weigh and transfer 5mg of Finasteride, 5mg of Tadalafil working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Acid degradation

Pipette 1 ml of above solution into a 10ml volumetric flask and 1 ml of 1N Hcl was added. Then,the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

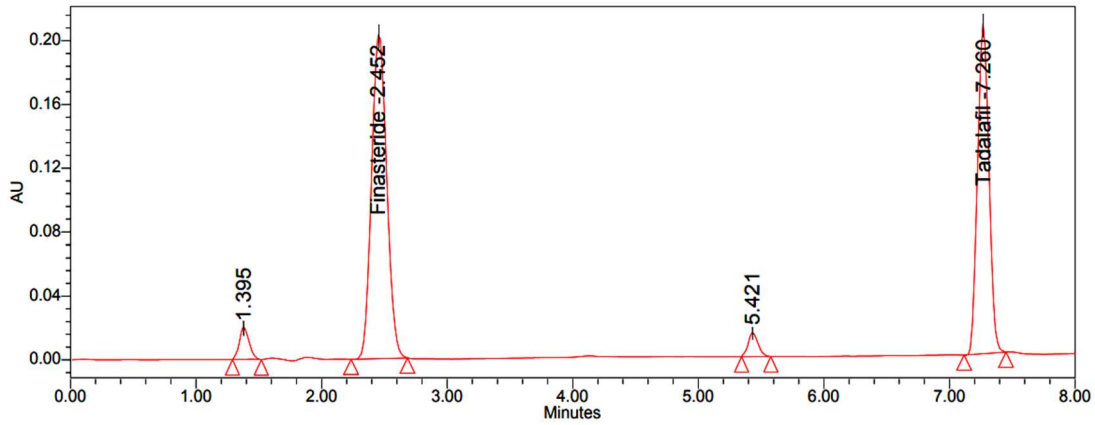

Figure 6: Chromatogram of Acid degradation

Alkali degradation

Pipette 1 ml of above solution into a 10ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N Hcl and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

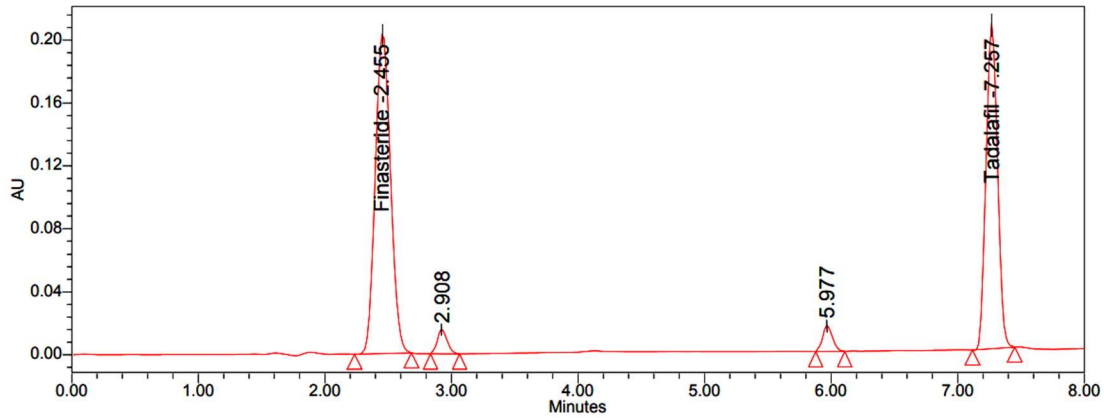

Figure 7: Chromatogram of Alkaline degradation

Peroxide degradation

Pipette 1 ml above stock solution into a 10ml volumetric flask, 1 ml of 3% v/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

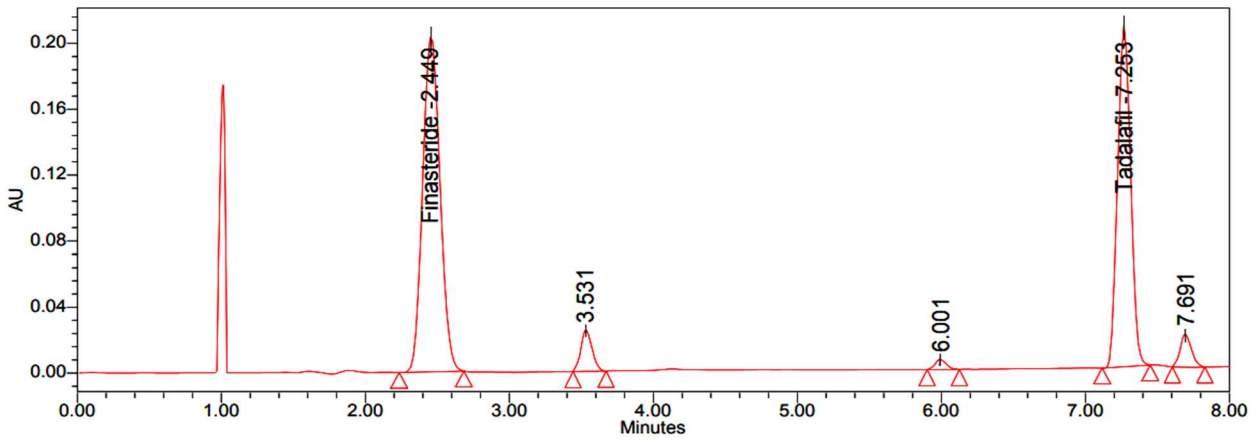

Figure 8 : Chromatogram of Peroxide degradation

Thermal induced degradation

Finasteride and Tadalafil samples were taken in Petridish and kept in Hot air oven at 110^0 C for 24hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

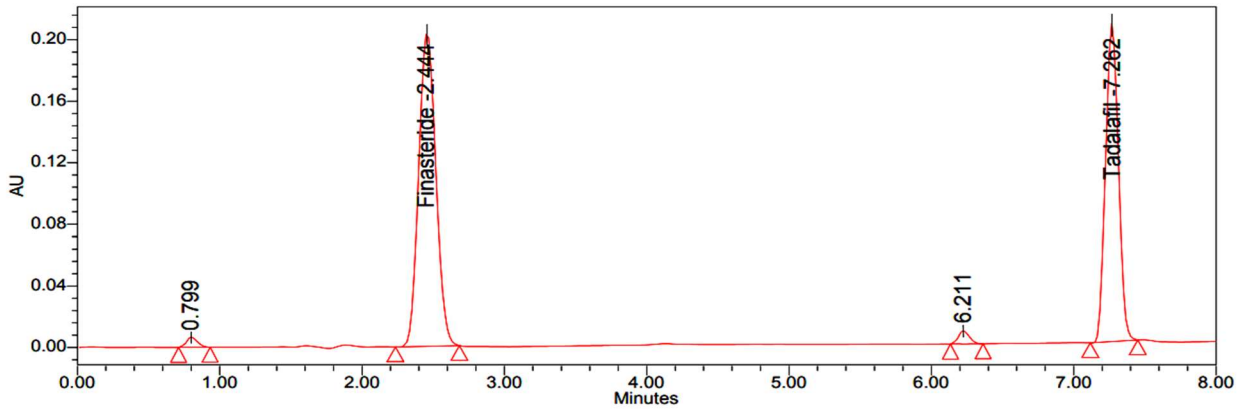

Figure 9 : Chromatogram of Thermal degradation

Hydrolysis degradation

Pipette 1ml above stock solution into a 10ml volumetric flask, 1 ml of water added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials

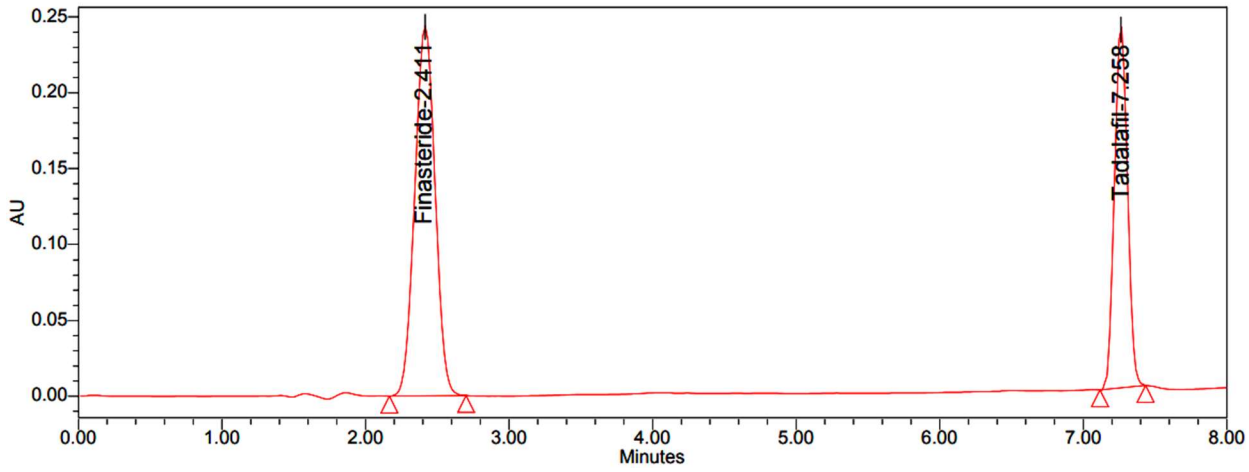

Figure 10: Chromatogram of Hydrolysis degradation

Table 9: Forced Degradation results for Finasteride and Tadalafil

Results: % Degradation results	Finasteride		Tadalafil	
	Area	% Degradation	Area	% Degradation
Control	2365530	0	2326806	0
Acid	2034763	14.0	2033670	12.6
Alkali	2052274	13.2	2017863	13.3
Peroxide	1979715	16.3	1959113	15.8
Thermal	2123289	10.2	2061184	11.4
Hydrolysis	2306377	2.5	2301412	1.1

CONCLUSION

The validated RP-HPLC-PDA method developed for the quantitative estimation of FSD and TDF in combination was evaluated for system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. All the validation results were within the specifications of the ICH guidelines. The developed method has proven to be rapid, accurate, and stability-indicating for the simultaneous determination of the combined FSD and TDF in the capsule dosage form in the presence of excipients and the degradation products and always a complete separation of both ingredients from their

degradation products and from the placebo. Therefore, this method can be employed in quality control to estimate the amount of FSD and TDF in bulk and in combined dosage forms.

Abbreviations

FSD: Finasteride

TDF: Tadalafil

Conflicts of interest

There are no conflicts to declare

ACKNOWLEDGEMENTS

The authors are thankful to Rakshith pharmaceuticals and Dr.Reddy's Laboratories, Hyderabad for providing gift samples of FSD and TDF and also to the NRI College of Pharmacy, Pothavarappadu for providing facilities to carry out the research work

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%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
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150%	3468324	7.5	7.44	99.2	

Table 5: Sensitivity parameters (LOD & LOQ)

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
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Table7:Robustness results of Finasteride by RP-HPLC

Parameter	Finasteride					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.8ml)	2.684	2553564		1.19	5444
	Actual (1ml)	2.415	2359707		1.17	5342
	More flow (1.2ml)	2.290	2194138		1.12	5279
Organic Phase change	Less Org (63:37)	2.700	2756501		1.16	5470
	Actual (70:30)	2.418	2380724		1.15	5355
	More Org (77:23)	2.225	1965248		1.08	5247

Table 8: Robustness results of Tadalafil by RP-HPLC

Parameter	Tadalafil					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.8ml)	7.685	2544562	23.01	1.13	5972
	Actual (1ml)	7.263	2325171	21.53	1.09	5869
	More flow (1.2ml)	6.958	2073124	22.68	1.06	5808
Organic	Less Org	7.711	2627541	23.18	1.14	5942

Phase change	(63:37)					
	Actual (70:30)	7.269	2339721	21.50	1.10	5862
	More Org (77:23)	6.606	1822874	21.30	1.04	5776

Table 9: Forced Degradation results for Finasteride and Tadalafil

Results: % Degradation results	Finasteride		Tadalafil	
	Area	% Degradation	Area	% Degradation
Control	2365530	0	2326806	0
Acid	2034763	14.0	2033670	12.6
Alkali	2052274	13.2	2017863	13.3
Peroxide	1979715	16.3	1959113	15.8
Thermal	2123289	10.2	2061184	11.4
Hydrolysis	2306377	2.5	2301412	1.1

Table 10: Results of the market product

Finasteride Label claim (mg)	Amount found(mg) ± SD (n=3)	% Assay ± SD (n=3)	Tadalafil Label claim (mg)	Amount found(mg) ± SD (n=3)	% Assay ± SD (n=3)
5	4.99 ± 0.08	99.85 ± 1.28	5	4.97± 0.14	99.42 ± 0.65
5	4.97 ± 0.05	98.64 ± 0.73	5	5.03 ± 0.27	100.2 ± 0.27
5	5.04 ± 0.10	100.82± 1.16	5	4.96 ± 0.35	99.24 ± 1.25