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# Exploring the Utility of HPTLC in Analysing Finasteride and Tadalafil: Methodology and Applications

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**ABSTRACT**

A new simple, precise, accurate and selective TLC-densitometry method has been developed for simultaneous determination of Finasteride and Tadalafil in tablet dosage form. Chromatographic separation was performed on aluminium plate precoated with silica gel 60 F 254 using toluene: n-propanol: triethylamine (6:2:2 v/v) as mobile phase. Detection was carried out densitometrically at 250 nm. The RF value of Finasteride and Tadalafil were 0.61 and 0.79, respectively. The reliability of the method was assessed by evaluation of linearity which was found to be 1000 – 5000 ng/spot for Finasteride and Tadalafil. Accuracy of the method was accessed by percentage recovery and found to be 99.77 ± 0.71 % for Finasteride and 99.75 ± 0.86 % for Tadalafil. The method can be used for routine analysis of Finasteride and Tadalafil in tablet dosage form.

**KEYWORDS:** Finasteride, Tadalafil, HPTLC, Simultaneous determination

## 1 INTRODUCTION

Finasteride is chemically N- (1,1-dimethylethyl) –3-oxo-4-aza-5K-androst-1-ene- 172- carboxamide1 (Fig. 1A). Finasteride is an antiandrogen agent (Male hormone) that was developed as drug for prostatitis. By inhibiting the enzyme 5a-reductase, Finasteride blocks male hormone testosterone from transferring into dihydro testosterone (DHT). Increased levels of (DHT) can lead to potential transcription of prostaglandin D2, which promotes the proliferate of prostate cancer cells. Tadalafil is mainly used for erectile dysfunction and pulmonary arterial hypertension. It works by inhibiting the enzyme phosphodiesterase type 5 and cyclic guanosine monophosphate. Chemically, Tadalafil is (6R-trans)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a- hexahydro-2-methyl-pyrazino (1',2':1,6.) pyrido (3,4-b) indole 1,4-dione and its empirical formula is $C_{22}H_{19}N_3O_4$ (Fig. 1B). Based totally there is no methods for estimation of Finasteride and Tadalafil for individual and different drug combination. High-performance thin-layer chromatography (HPTLC) method for the estimation of Finasteride and Tadalafil in bulk and tablet dosage form has been reported. The HPTLC method provides accurate and precise results which are comparable to that of liquid chromatographic method. Reduced sample preparation methods, less analysis time, and small quantity of mobile phase required are some of its advantages over liquid chromatography. Densitometric scanning used in HPTLC for quantitative analysis offers advantage of accuracy, precision, and specificity over conventional methods used in TLC. Stability and degradation samples can also be analyzed using a densitometer. So, the present study involves development and validation of the stability-indicating high-performance thin-layer chromatographic method for the estimation of Finasteride and Tadalafil in tablet formulation. Structure of Finasteride and Tadalafil was shown in Fig.1.

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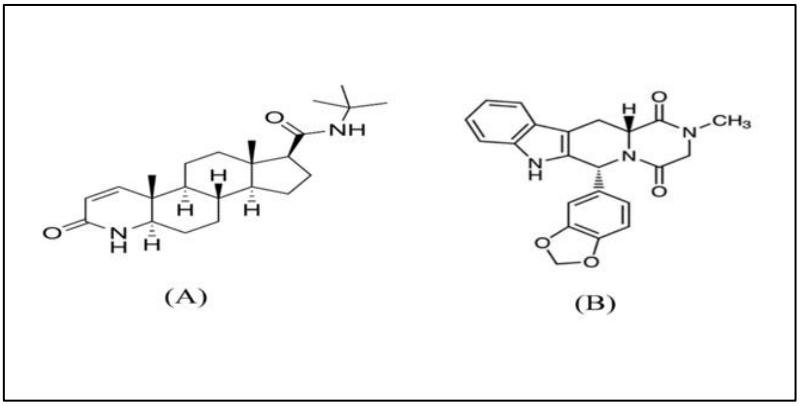


**Fig.1** Chemical Drug Compounds Structure of Finasteride (A), Tadalafil (B)

## 2. MATERIALS&METHODS

### 2.1 HPTLC instruments

For the chromatographic measurements Camag HPTLC, instrument containing Linomat V sample applicator was used for the study. Aluminum HPTLC plates (10 cm × 10 cm), precoated with silica gel 60F 254 were used as stationary phase. Densitometric scanning of developed densitograms were performed using Camag TLC scanner. The CAMAG winCATS software was used for further data analysis.

### 2.2 Chemicals and reagents

Analytically pure Finasteride and Tadalafil was obtained as gift sample from Reputed Pharmaceutical company. Acetonitrile (HPLC grade) was procured from Merck laboratories. Toluene (HPLC grade), n- Propanol and trimethylamine were procured from Loba Chemie Pvt. Ltd., India.

### 2.3 Chromatographic conditions:

The samples were applied in the form of band with width 6mm on pre-coated silica gel aluminum plate 60F-254 with 250mm thickness, and distance of 10mm from bottom with Linomat sample applicator equipped with 100µL Hamilton syringe. The plates were prewashed by methanol and activated at 60˚C for 5 min prior to chromatography. The standard and sample solutions were applied with 100nL/s; at 5mm space between two bands under a stream of nitrogen. The elution solvent consists of Toluene: isopropyl alcohol: Triethylamine (6: 2: 2 v/v).Linear ascending development was carried out to distance of 8cm in 20/10cm twin trough TLC developing chamber CAMAG at room temperature and previously saturated with mobile phase for 30 min. TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on CAMAG TLC scanner III in the absorbance mode at 250nm. The source of radiation utilized was helium lamp.

### 2.4 Mobile phase and development

Linear ascending chromatographic development was performed using mobile phase Toluene: isopropyl alcohol: Triethylamine (6: 2: 2 v/v/v). The development was carried out in twin-trough glass chamber previously equilibrated with the mobile phase for 30 min. The mobile phase was allowed to migrate a distance of 80 mm, and after development, plates were removed and dried.

### 2.5 Densitometric analysis

The developed TLC plates were scanned in the reflectance mode using winCATS software. The light source used was ileum lamp, and bands were scanned at 250 nm. The dimension of the slit was 5 mm in length and 0.45 mm in width. Peak area and peak height data were obtained for each developed band, and regression equation was developed by plotting peak areas versus concentration.

**2.6 Preparation of standard stock solution**

Finasteride and Tadalafil (10 mg) were weighed accurately and transferred to 10-mL volumetric flasks and dissolved in a few millilitres of acetonitrile and sonicated for 5 min. Solution was diluted up to the mark with acetonitrile which gave a concentration of 1000 µg/mL. Finasteride 1000 ng and Tadalafil 3000 ng was shown in Fig.2&3. Mixture and Formulation of Finasteride and Tadalafil was shown in Fig 4&5.

**3. Validation**

International Conference on Harmonization (ICH) published a guideline Q2 (R1) for the validation of analytical method. The developed method was validated with respect to linearity, accuracy, precision, specificity, and robustness.

**3.1 Calibration curve**

The stocks solution of 1000µg/ml was prepared in acetonitrile. The different volumes of intermediate stock 1, 2, 3, 4, and 5 µl were applied on plate to obtain a concentration of 1000, 2000, 3000, 4000 and 5000ng /Spot. The calibration curve was plotted using peak area against the concentration of drug and the linearity data was treated by the linear least square regression.

**3.2 Precision:**

Repeatability of sample application and measurement of peak areas were carried out using six replicates of the same spot. The intra-day and inter-day variation for the determination of Finasteride and Tadalafil was carried out at a concentration of 2000, 4000 and 6000ng /spot.

**3.3 Accuracy**

The accuracy is the closeness of test result to the true value. To perform accuracy, recovery studies were carried out. Known amount of Finasteride and Tadalafil was spiked at three concentration levels (50, 100, and 150%) to a pre quantified formulation. The solutions were diluted and analyzed by developing densitograms using optimized chromatographic conditions. The peak area was noted, and the amount of Finasteride and Tadalafil was estimated with the help of regression equation.

**3.4 Repeatability**

Repeatability of the method was assessed by applying band of Finasteride and Tadalafil (4000 ng/band) six times on an HPTLC plate. The plates were developed, and peak areas were determined. To study the scanner repeatability, the same spot was scanned six times, peak area was determined, and variability in the result was analyzed.

**3.5 Sensitivity**

The lowest amount of analyte that can be detected in a method is called limit of detection (LOD), and the lowest amount of anlayte that can be quantified is called limit of quantification (LOQ). LOD and LOQ were calculated using the following equation as per ICH guidelines.

$$LOD = 3.3*(SD/Slope)$$

$$LOQ = 10*(SD/slope)$$

where σ is the standard deviation of y-intercepts of regression lines, and S is the slope of the calibration curve.

**3.6 Robustness**

Small deliberate changes were introduced in the method to assess the robustness of the method. Changes in mobile phase ratio and chamber saturation time were introduced, and the effects on densitogram were analyzed. The study was performed triplicate and %RSD was calculated.

**4. Forced degradation study**

To find out the intrinsic stability of the drug molecule and possible degradation pathway, forced degradation studies were performed as per ICH guideline Q1A (R2) and Q1B using different stress conditions. Stress studies were performed using acid and base hydrolysis, oxidative hydrolysis, thermal degradation, and UV light exposure conditions.

**4.1 Acid hydrolysis**

To perform base hydrolysis, 10 mg of Finasteride and Tadalafil in 10 ml volumetric flask to add 1ml of 0.1 M hydrochloric acid and make up the volume with acetonitrile refused at 40°C for 30 mins. After completion of 30 mins, above the solution were applied on TLC plates in triplicates and corresponding concentrations were obtained (Fig.6)**.**

**4.2 Alkali hydrolysis**

To perform base hydrolysis, 10 mg of Finasteride and Tadalafil in 10 ml volumetric flask to add 1ml of 0.1 M Sodium hydroxide and make up the volume with acetonitrile then refluxed at 40°C for 30 mins. After completion of 30 mins, above the solution were applied on TLC plates in triplicates and corresponding concentrations were obtained. The solution was analyzed and developed; densitogram showed complete degradation of Finasteride and Tadalafil (Fig.7).

**4.3 Oxidative stress degradation**

To perform oxidative stress degradation, 10 mg of 10 mg of Finasteride and Tadalafil in 10 ml volumetric flask to add 1ml of 0.1 M 30% hydrogen peroxide and make up the volume with acetonitrile then refluxed at 40°C for 30 mins. After completion of 30 mins, above the solution were applied on TLC plates in triplicates and corresponding concentrations were obtained (Fig .8).

**4.4 Thermal degradation**

To perform dry heat degradation study, pure drug samples of Finasteride and Tadalafil was exposed in the oven at 70 °C for 2 h. It was cooled, and drug was weighed (10 mg) and transferred to 10-mL volumetric flask. It was dissolved and diluted up to the mark using acetonitrile (Fig.9).

**4.5 Photo degradation**

Analytically pure samples of Finasteride and Tadalafil were exposed to UV light for 24 h. A 10 mg of drug was weighed and transferred to a 10-mL volumetric flask. It was dissolved and diluted up to the mark using acetonitrile.

All the reaction solutions were applied using applicator microliter syringe on TLC plates. Plates were developed using optimized chromatographic conditions, and densitograms were recorded (Fig.10).

**4.6 Analysis of marketed formulation**

Twenty tablets were weighed and powdered. The powder equivalent to the 25 mg of Finasteride and Tadalafil was weighed and transferred to the 50-mL volumetric flask. A small amount of (10 mL) acetonitrile was added to the above volumetric flask and sonicated for 10 min. The solution was filtered using Whatman filter paper No. 45, and the volume was made up to the mark with acetonitrile (500 µg/mL). Using a sample applicator, 8 µl of sample was applied on the stationary phase which gave 4000 ng/band concentration of Finasteride and Tadalafil. The stationary phase plates were developed as per optimized chromatographic conditions and scanned. The areas were determined, and quantification was carried out by keeping this value in regression equation.

**4.7 RESULTS**

The thin-layer chromatographic method was developed and validated for the analysis of Finasteride and Tadalafil .The stationary phase used was silica gel F254 precoated aluminium plates, and optimized mobile phase selected for the analysis was a mixture of Toluene: isopropyl alcohol: Triethylamine (6: 2: 2 v/v). To select the detection wavelength, developed plate was subjected to densitometric measurements in scanning mode in the UV region of 400–200 nm, and the overlaid spectrum was recorded using CAMAG TLC Scanner. The overlaid spectra showed that drug was absorbed appreciably at 250 nm. The optimized conditions gave

the compact band of Finasteride and Tadalafil with retardation factor (Rf) value of 0.61 and 0.79. The mobile phase chamber was saturated with the mobile phase for 30 min, and solvent was allowed to migrate to a distance of 80 mm. The calibration curve of the both drug was found to be linear in the range of 1000–5000 µg/band with a linear correlation coefficient (r 2) 0.9979. Similarly, interday precision was carried out by measuring response for 3 consecutive days, and %RSD was found to be 1.13– 1.99%. Repeatability study was performed by scanner repeatability study and injection repeatability study. Drug solution (4000 ng/band) was applied, analyzed six times and percentage RSD value of the response was found to be less than 1%.

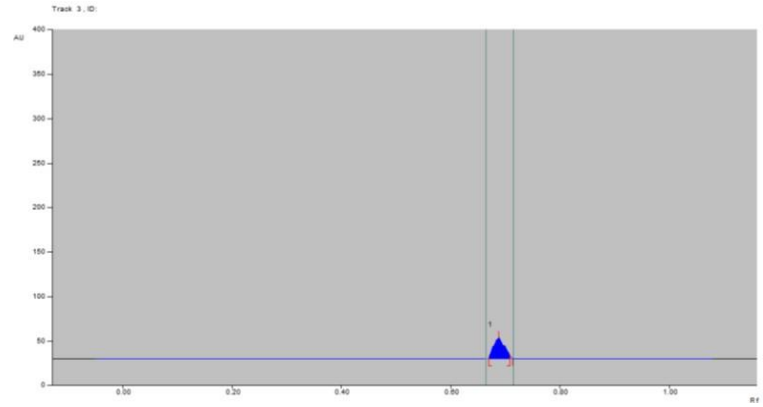

**Fig.2 Finasteride 1000ng**

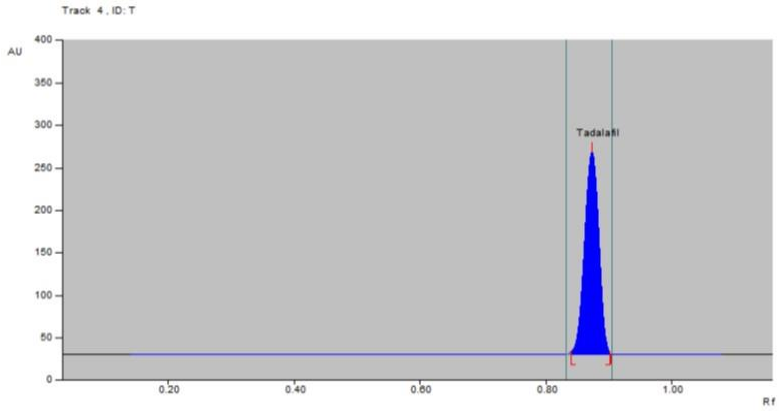


**Fig.3 Tadalafil 3000ng**

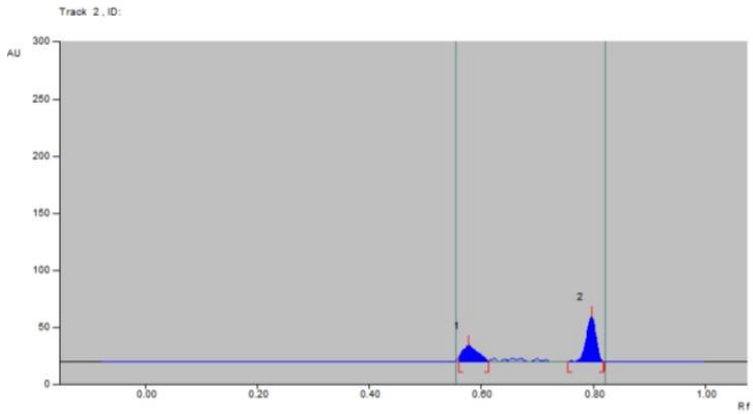


**Fig.4 Mixture form**

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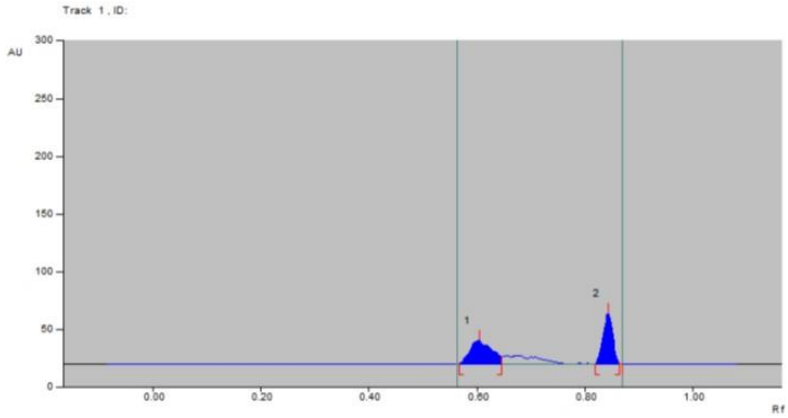


**Fig.5 Formulation**

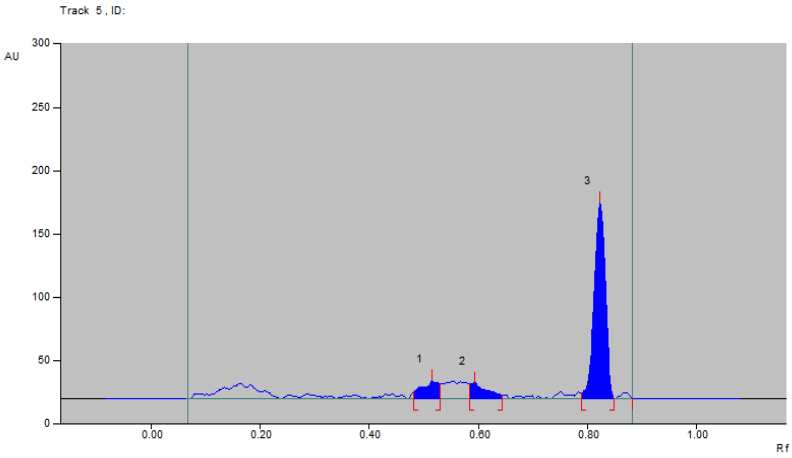


**Fig.6 Acid Degradation study**

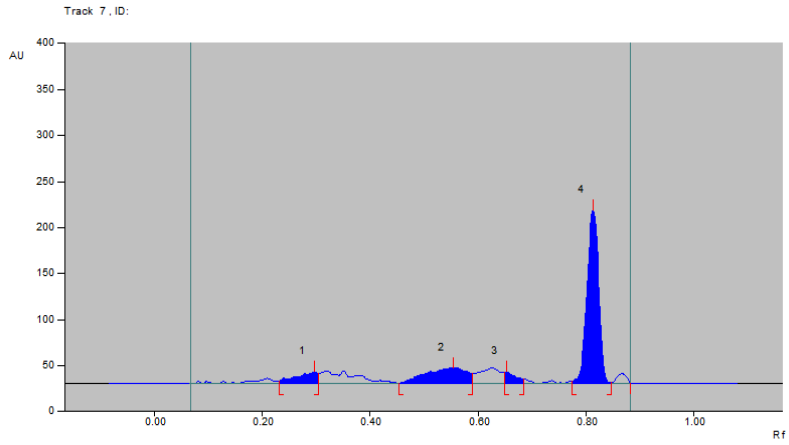


**Fig.7 Base Degradation study**

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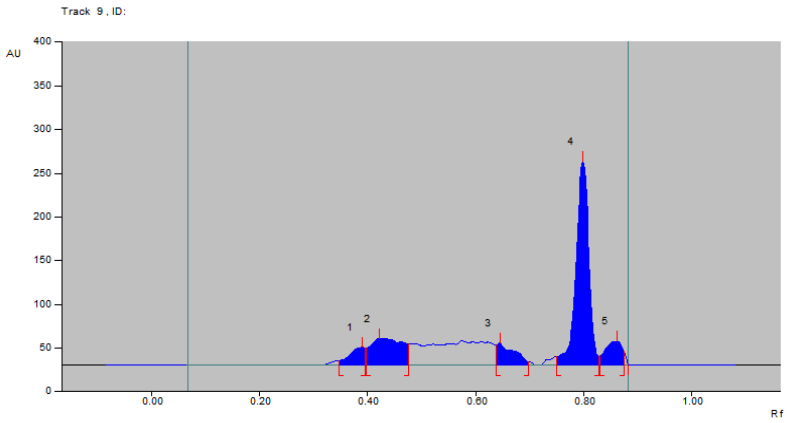


**Fig.8 Hydrogen peroxide-induced degradation**

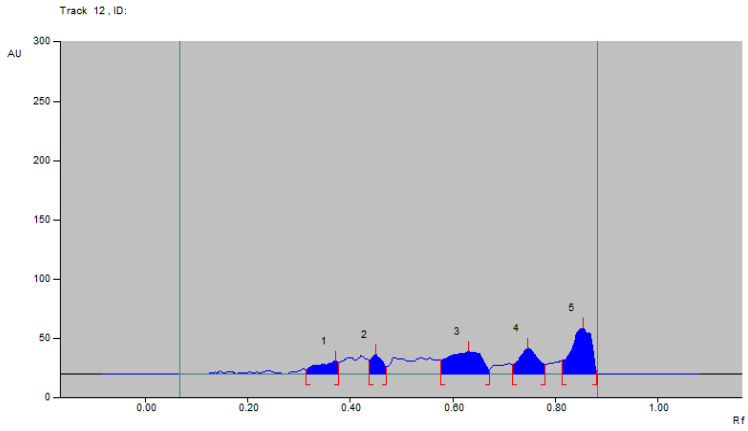


**Fig.9 Light heat degradation**

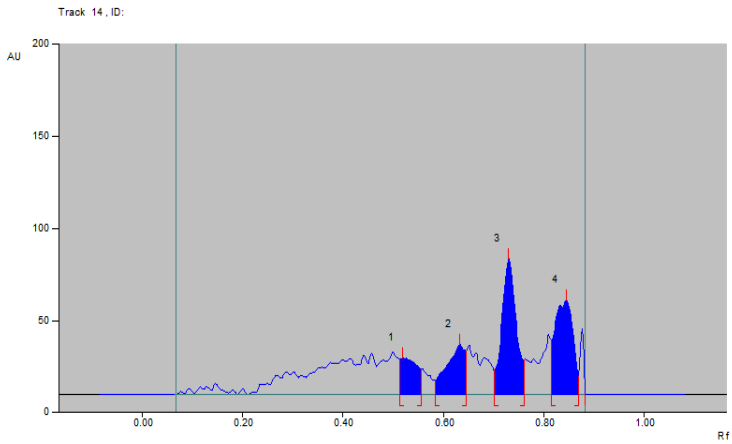


**Fig. 10 Dry heat degradation**

## 5. Validation of the Method

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to linearity and range, specificity, precision, accuracy, limit of detection and limit of quantification.

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### 5.1 Linearity and Range

A stock standard solution was prepared for both Finasteride and Tadalafil and they were serially diluted to yield five standard solutions. The calibration plots revealed good linear relationships between area and concentration over the range of 1000- 5000 ng/spot for Finasteride and Tadalafil. The standard curves for Finasteride and Tadalafil are shown in **(Fig.11& 12) (Table 1).**

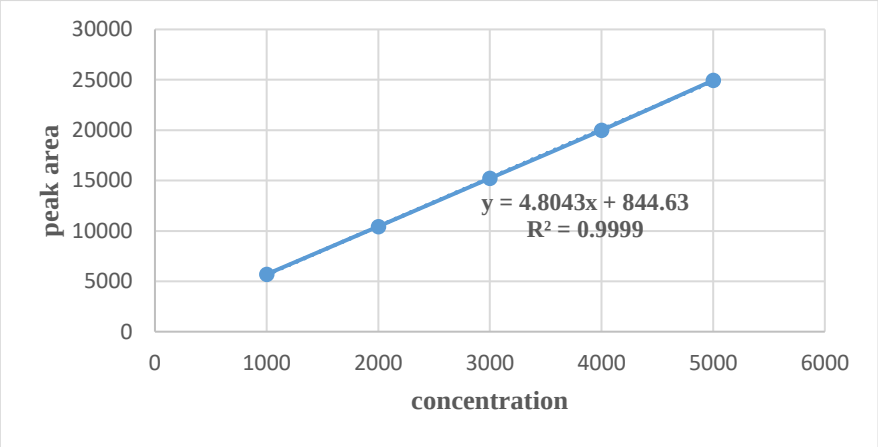


**Fig.11 Linearity graph of Finasteride**

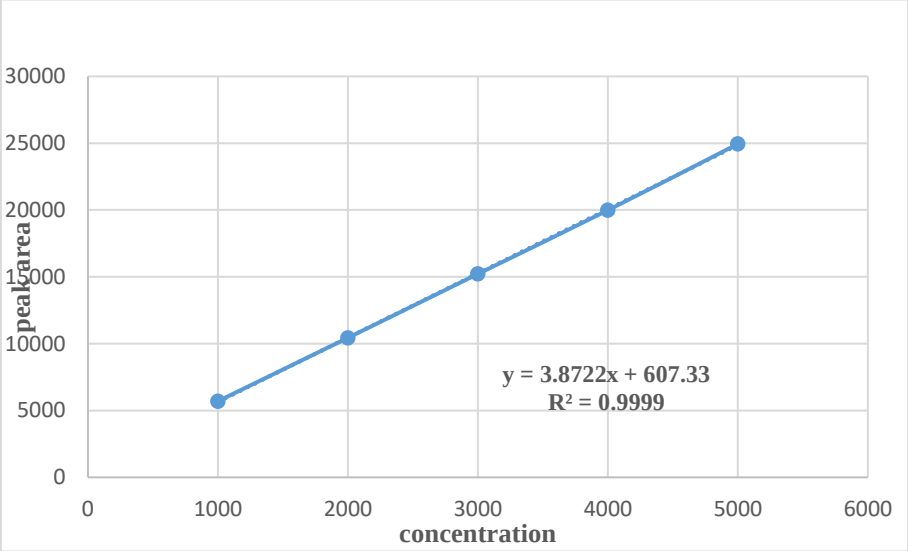


**Fig.12 Linearity graph of Tadalafil**

**Table: 1 Linearity of Finasteride and Tadalafil**

| S.No | Conc of Finasteride & Tadalafil (ng/Spot) | Peak area | |
|---|---|---|---|
| | | Finasteride | Tadalafil |
| 1 | 1000 | 5697.35 | 4531.56 |
| 2 | 2000 | 10439.16 | 8351.34 |
| 3 | 3000 | 15219.55 | 12119.23 |
| 4 | 4000 | 19987.29 | 16097.57 |
| 5 | 5000 | 24945.03 | 20019.67 |

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**5.2 Precision**

**A) Intraday Precision**

Intra-day precision was found out by carrying out the analysis of the standard drug three different concentrations of 1000,2000,3000ng for Finasteride, 1000,2000,3000 ng for Tadalafil were selected from linearity range. Intraday analysis was carried on same day in three replicates. Each concentration was applied in duplicate and RSD was calculated and the results shown in **(Table.2)**.

**Table:2 Intraday studies**

| No. of Injection | Conc of FIN (ng/Spot) | Peak area* | % RSD | Conc of TAD (µg/ml) | Peak Area | % RSD |
|---|---|---|---|---|---|---|
| 6 | 3000 | 15220.25 | 0.38 | 3000 | 16098.55 | 0.47 |

**B) Interday Precision**

Inter-day precision was found out by carrying out the analysis of the standard drug at three different concentrations of 3000ng for Finasteride, and 3000 ng for Tadalafil were selected from linearity range. Interday analysis was carried on three different days in three replicates. Each concentration was applied in duplicate and RSD was calculated and the results Shown in **(Table.3)**.

**Table:3 Interday studies**

| Day | Conc of FIN (ng/Spot) | Peak Area | % RSD | Conc of TAD (ng/Spot) | Peak Area | % RSD |
|---|---|---|---|---|---|---|
| | | | | | | |
| Day1 | 3000 | 15219.59 | 0.34 | 3000 | 16097.29 | 0.42 |
| Day2 | 3000 | 15220.50 | 0.39 | 6 | 16098.35 | 0.49 |
| Day3 | 3000 | 15217.35 | 0.37 | 6 | 16099.85 | 0.44 |

**C) Repeatability**

Repeatability was determined by applying the corresponding microlitre of standard Solution containing 3000 ng/spot of Finasteride and 4000 ng /spot of Tadalafil in six replicates and Respective areas were calculated. Statistical data of the regression equation are shown in **(Table.4)**.

**Table:4 Statistical data of the regression equation**

| Parameters | Finasteride | Tadalafil |
|---|---|---|
| Linearity ranges (ng/spot) | 1000-5000 | 1000-5000 |
| Coefficient of correlation | 0.999 | 0.999 |
| Slope | 4.8043 | 3.8722 |
| y- intercept | 844.63 | 607.33 |

**Limits of Detection and Quantification**

The limit of detection was found to be 21.63 ng /spot and 129.91 ng/spot for Finasteride and Tadalafil, respectively. The limit of quantification was found to be 65.55 ng/spot and 393.68 ng/spot for Finasteride and Tadalafil respectively, which was lower than that reported earlier.

## 6. CONCLUSION

A new HPTLC method was used for the stability-indicating simultaneous determination of Finasteride and Tadalafil in pharmaceutical dosage form has been developed. The mobile phase system consisting of Toluene: n-propanal: Tri ethylamine in the ratio of 6:2:2 v/v/v was used. $R_f$ values of Finasteride and Tadalafil were found to be 0.61 and 0.79, respectively. The forced degradation studies Finasteride and Tadalafil were performed on it was subjected to stress conditions which include Acid (0.1 M HCl), Base (0.1M NaOH), Thermal (50°C), Oxidation (30% H2O2) and Photolytic (exposure to light). The calibration curve was plotted using the peak area of standard drugs Vs concentration of the standard solution. Both calibration curves of Finasteride and Tadalafil were linear with $R^2$ values of 0.999 and 0.999. The experiment revealed degradation under these conditions, highlighting the method's efficacy in separating the main drug peaks from the degradation product peaks. This characteristic qualifies it as a stability-indicating method, offering valuable insights into the integrity of the drugs over time.

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Conflict of Interest Statement: All authors have nothing else to disclose.