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DEVELOPMENT AND VALIDATION OF A NOVEL STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF TADALAFIL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

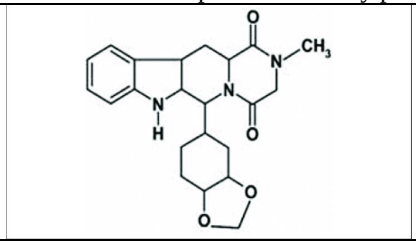
A reliable and selective (RP-HPLC method was developed for the detection of tadalafil in tablet dosage forms. The study was conducted using a ZORBAX C18 non-endcapped column with a particle size of 5 µm and dimensions of 250 mm × 4.6 mm. Chromatographic separation under isocratic conditions was achieved using a mobile phase made up of methanol and acetate buffer in a 85:15 (v/v) ratio. The mobile phase was sonicated to remove gas before being run through a 0.45 µm membrane filter. The detecting wavelength was 284 nm, and the flow rate was maintained at 1 mL/min. In these conditions, the retention period of tadalafil was approximately 3.61 minutes.. The developed approach was validated in accordance with recognized standards by assessing elements such as linearity, accuracy, precision, and robustness. Linearity was seen in the concentration range of 0.5 to 200 µg/mL. With a mean recovery of about 99.2% and (RSD) values less than 2%, the method demonstrated good accuracy. Precision was confirmed in both intra- and inter-day studies, and strong reproducibility was demonstrated by RSD values that were consistently less than 2%. Robustness testing confirmed the method's reliability with deliberate, small changes in analytical conditions. Additionally, studies on forced deterioration were conducted in a variety of stress environments, including neutral, acidic, alkaline, photolytic, and oxidative ones. Under these circumstances, tadalafil showed signs of degradation, and the resulting degradation products were identified and monitored using the standard methodology. All things considered, this straightforward and effective method can be used to regularly test tadalafil in pharmaceutical formulations. Additionally, by providing useful details about the drug's stability profile, the degradation test results improve its use in quality control and stability testing prog.

KEYWORDS: Tadalafil, HPLC, Force degradation etc.

INTRODUCTION

Tadalafil is a crystalline powder that ranges from white to off white. It is soluable in methanol and just weakly soluble in water. Tadalafil has a molecular weight of 438.4 g/mol and the chemical formula is C22H19N3O4. Tadalafil is quickly absorbed when taken orally. Within 30 to 120 minutes, the plasma concentration reaches its maximum. The cytochrome P450 enzyme system breaks down tadalafil in the liver. 17.5 hours is the elimination half-life. One type of PDE5 inhibitor is tadalafil. One enzyme that degrades cGMP is PDE5. One signaling molecule involved in erectile function is cGMP. Tadalafil helps to raise the amounts of cGMP in the penis, which results in an erection, by preventing the breakdown of cGMP.

Table 1: Drug profile for Tadalafil.

Drug Name	**Tadalafil**
Brand Name	Cialis
Class	Phosphodiesterase type 5 (PDE5) inhibitor
Indications	Erectile dysfunction, benign prostatic hyperplasia (BPH)
Dosage	2.5-20 mg orally once daily
Contraindications	Heart disease, liver disease, kidney disease, nitrate medications
Precautions	Use with caution in people with diabetes, high blood pressure, and bleeding disorders
Overdose	Symptoms may include headache, flusing and upset stomac seek inedical attention if you experience any of these symptoms
Storage	Store at room temperature in a dry place
Structure	
IUPAC	(6R,12Ar)-6-(1,3-Benzodioxol-5yl)-2-methy1-2,3,6,7,12a-hexahydropyrazino[1’,2’:1,6]pyrido[3,4-b]bindole-1,4-dione.

EXPERIMENTAL

A UV spectrophotometer with a PDA detector, specifically the Alliance model 2996, was employed in this study to ascertain the λ max values of the pharmaceutical compounds. A non-endcapped ZORBAX C18 column (250 x 4.6 mm, 5 µm) was make use of for the purpose of method development. The chromatographic system was operated under the control of Empower analytical software.

Accurately weighing 10 mg of Tadalafil and dissolving it in a 25 mL volumetric flask with HPLC-grade methanol to reach a concentration of 100 µg/mL produced a stock solution. The mobile phase was used to further dilute this solution, which was then filtered. Operating under isocratic method, the mobile phase was a 15:85 (v/v) combination of acetate buffer and methanol Before being used, the mobile phase was filtered through a 0.45 µm membrane filter and degassed using sonication.

Method Validation

Precision, accuracy, linearity, specificity, LOD, LOQ, and robustness were all assessed in order to validate the procedure. Twenty microliters of each solution (n=3) were injected into the HPLC system after 0.5 to 200 µg/mL Tadalafil solutions were made from the stock on dilution with mobile phase. The chromatogram's peak area was identified. Concentration was plotted against mean peak area on the calibration graph. Following the ICH rules,

intraday and interday precision tests were conducted on the same day and three days in a row, respectively. The standard addition method was used to accomplish the method's accuracy, and intentional changes were made to the optimal parameters, including the pH (± 0.2 units), mobile phase ratio (± 2%), flow rate (± 0.1 mL), and detection wave length (282 nm & 286 nm), to ensure robustness. Key criteria such as precision, accuracy, linearity, specificity, (LOD), (LOQ), and robustness were evaluated in order to validate the analytical method. The stock solution was diluted with the mobile phase to create tadalafil solutions with concentrations ranging from 0.5 to 200 µg/mL. The average peak area for each concentration was measured after three separate injections of an aliquot of 20 µL from each concentration into the HPLC apparatus. The mean peak area was plotted against the matching concentration to create a calibration curve. In compliance with ICH recommendations, intraday and interday studies were used to assess precision; the former were carried out across three consecutive days, while the latter were carried out inside a single day. The usual addition methodology was used to assess the correctness of the method. In order to assess robustness, some analytical parameters were purposefully changed, such as the detection wavelength (tested at 282 nm and 286 nm), pH (±0.2 units), mobile phase composition (±2%), and flow rate (±0.1 mL/min).

Experimental Applicability To Tadalafil Tablet

Two distinct commercial brands of tadalafil tablets were purchased from a pharmacy. Methanol was used to extract 10 mg of Tadalafil, and then the mixture was sonicated. The mobile phase was then added to the resultant solution after it had been filtered. The average peak area was taken from the corresponding chromatograms after each sample (n=3) was injected into the chromatographic apparatus for analysis.

Forced degradation studies

Tadalafil was subjected to forced degradation investigations under a range of stress settings in order to assess its stability. Prior to being diluted with the mobile phase, the drug solutions were neutralized for both acidic and alkaline degradation. 30% v/v hydrogen peroxide was used for oxidative stress tests. Experiments on photolytic and thermal deterioration were also conducted. 20 µL of each treated sample was injected into the chromatographic apparatus for analysis, and the chromatograms' corresponding peak regions were noted.

RESULTS AND DISCUSSION

Optimization of methods While the approach was being developed, Initially, a 250 x 4.6 mm, 5 µm non-endcapped ZORBAX C18 column was utilized with a The mobile phase was a 15:85 (v/v) acetate buffer and methanol mixture that flowed at a rate of 1 milliliter per minute under isocratic conditions. Table 2 displayed the ideal chromatographic conditions. Chromatograms were displayed in Figure 2.

Table 2: Tadalafil's ideal chromatographic conditions.

Parameter	Optimized chromatographic conditions
Detector	photodiode array detector
Flow rate	1.0 mL/min
UV detection	284
Column temp.	25 ± 2°C
Injection volume	20 µL
Elution	Isocratic mode
Mobile phase M	mobile phase made up of acetate buffer and methanol in a 15:85 (v/v) ratio
Column	A non-endcapped ZORBAX C18 column (250 x 4.6 mm, 5 µm)
Retention time	3.61
Total run time	7 min

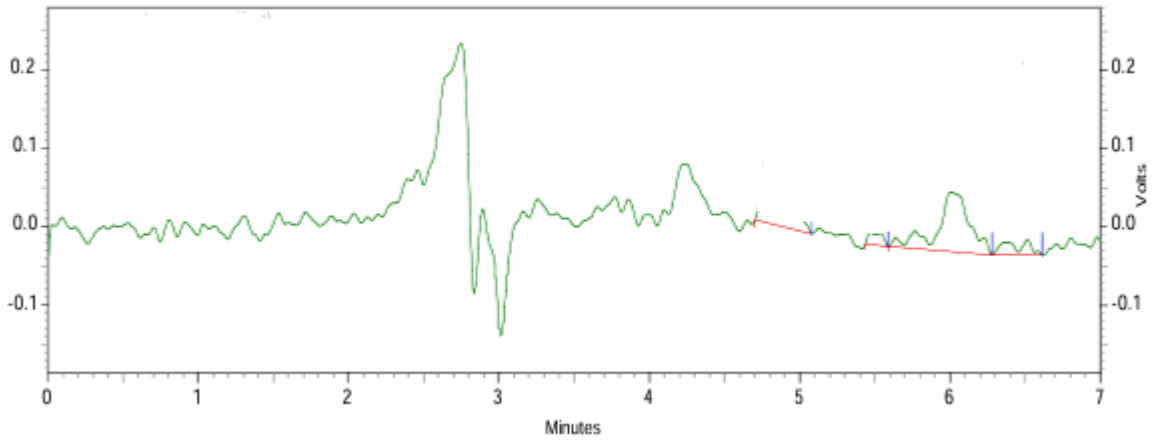


Fig. 1: Typical Chromatogram for Blank.

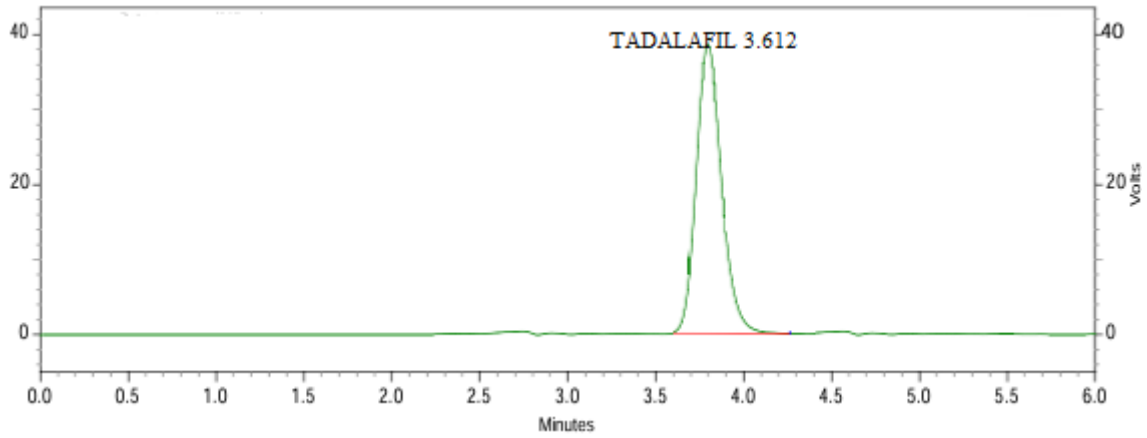


Fig. 2: Typical Chromatogram for Tadalafil standard (20 µg/mL).

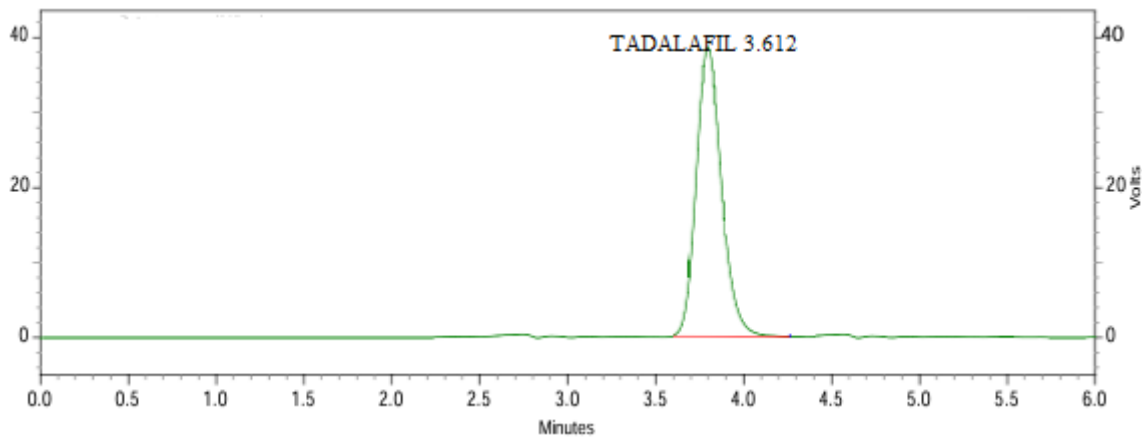


Fig. 3: Typical Chromatogram For Tadalafil sample (20 µg/mL).

Method Validation

To develop a range of Tadalafil standard solutions with concentrations between 0.5 and 200 µg/mL, the stock solution was diluted with the mobile phase. Each generated solution was sampled with 20 µL for the HPLC system. A calibration curve is made by plotting the connection between the Tadalafil concentration (µg/mL) on the x-axis and the appropriate peak area on the y-axis. The medication will react linearly within the tested concentration range, according

to Beer-Lambert's law. As seen in Table 3 and Figure 4, the findings showed excellent linearity with a regression equation of y = 369958x -1 E +06 and a correlation coefficient (r2) of 0.8828.

Table 3: Linearity study of of Tadalafil.

Conc. (µg/mL)	*Mean peak area	% RSD
0.5	10848	0.21
1	21899	0.14
2	42440	0.50
5	103520	0.70
10	200728	1.18
20	392988	1.38
50	1014718	0.12
80	1658888	0.58
100	2075257	0.21
120	2490678	0.41
150	3155755	0.53
200	4157278	0.69

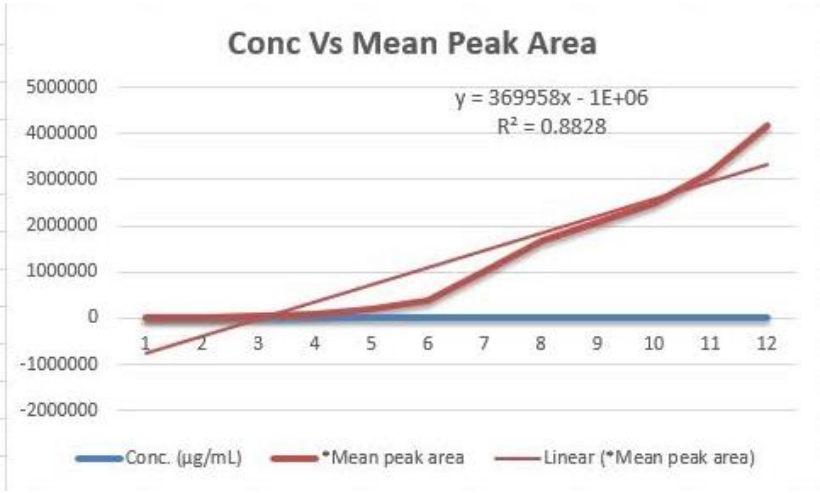


Figure 4: Linearity plot of Tadalafil.

The results showed that the limit of quantification (LOQ) was 0.411 µg/mL and the limit of detection (LOD) was 0.1321 µg/mL. Both intraday and interday variation precision was assessed. Table 4 shows that the intraday relative standard deviation (%RSD) was between 0.098% and 0.62%, while Table 6.4 shows that the interday %RSD was between 0.17% and 0.21%. When accuracy was evaluated, the percentage RSD values ranged from 0.60 to 1.38% (refer to Table 6).

Table 4: Intraday precision study of Tadalafil.

Conc. (µg/ml)	*Mean Peak Area	Statistical Analysis
		*Mean peak area ± SD (% RSD)
20	392985	392705 ±2458.67(0.62)
20	390118	
20	395012	
50	1013718	1014671 ±4427.67(0.43)
50	1019498	
50	1010798	
100	2074262	2072011 ±2049.66(0.0.098)
100	2071520	
100	2070252	

Table 5: Inter day precision study of Tadalafil.

Conc. (µg/ml)	*Mean Peak Area			*Mean peak area ± SD (% RSD)
	Day 1	Day 2	Day 3	
20	382462	381261	381189	381637.3±715.089 (0.18)
50	1002467	1001561	1001434	1001821±563.33 (0.056)
100	20506276	20536145	20543456	20528634±19697.20 (0.0595)

*Mean of three replicates

Table 6: Accuracy study of Tadalafil.

Conc. (µg/ml)		Conc. (µg/ml)		*Mean conc. (µg/mL) ± SD (% RSD)	% Recovery
Formulation	Pure drug	Total	Obtained		
10	5	15	15.1	15.33 ± 0.20 (1.38)	
10	5	15	14.8		
10	5	15	15.2		
10	10	20	20.1	20.06 ± 0.15 (0.76)	
10	10	20	19.9		
10	10	20	20.2		
10	15	25	25.1	25.066 ± 0.15 (0.60)	
10	15	25	24.9		
10	15	25	25.2		

***Mean of three replicates**

Table 7: Robustness conditions of Tadalafil.

Parameter	Condition	*Mean peak area	%RSD
Flow rate (mL/min)	0.9	389058	0.88
	1.00	382345	
	1.1	386789	
Detection wavelength (± 2 nm)	282	388678	0.64
	284	389067	
	286	384534	
Mobile phase (v/v) Acetate buffer and methanol in a 15:85 (v/v) ratio,	20:80	389980	0.11
	15:85	389089	
	10:90	389767	

***Mean of three replicates**

Experimental applicability to Tadalafil Tablet

"The purity of Tadalafil in the marketed formulations was consistently found to be 99.00 AND 99.20 as shown in Table 8. No interference from excipients was observed, as illustrated in Figure 3."

Table 8: Assay of Tadalafil Tablet.

Brand	Lable claime (mg)	Amount found mg	*Recovery (%)
I	10	9.90	99.00
I	10	9.92	99.20

***Mean of three replicates**

Forced degradation studies

Tadalafil exhibited less than 20% degradation under all stress conditions, suggesting a high level of stability. The chromatographic profiles obtained during the stress studies are presented in Figures 5 through 9. Additionally, system suitability parameters remained within the predefined acceptance limits, as summarized in Table 9.

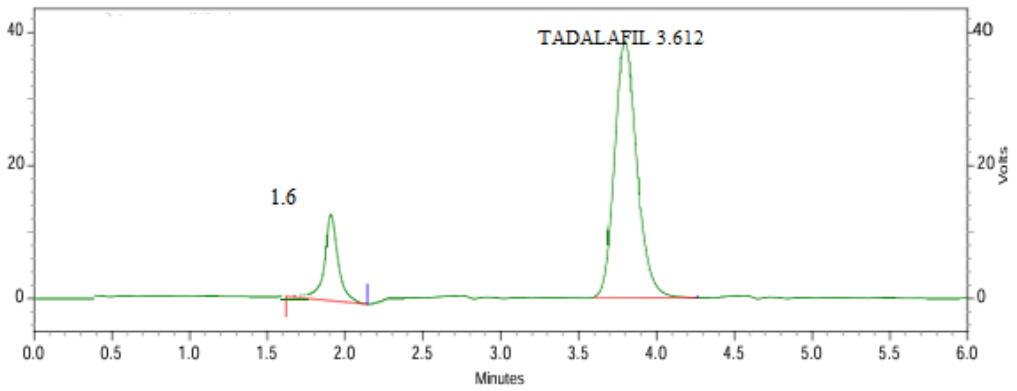


Fig. 5: Chromatogram for Tadalafil at acidic degradation.

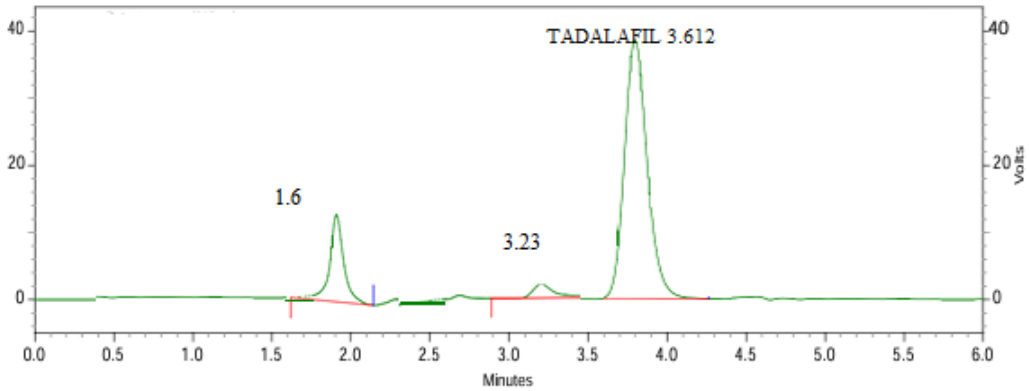


Fig. 6: Chromatogram for Tadalafil at alkaline degradation.

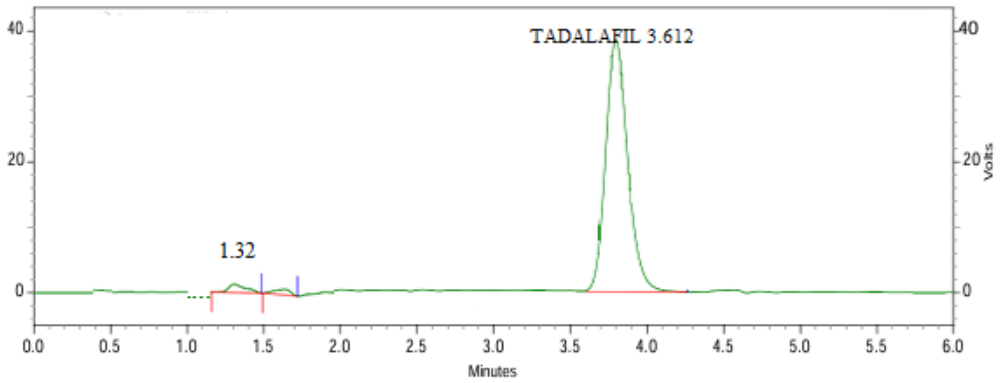


Fig. 7: Chromatogram for Tadalafil at oxidative degradation.

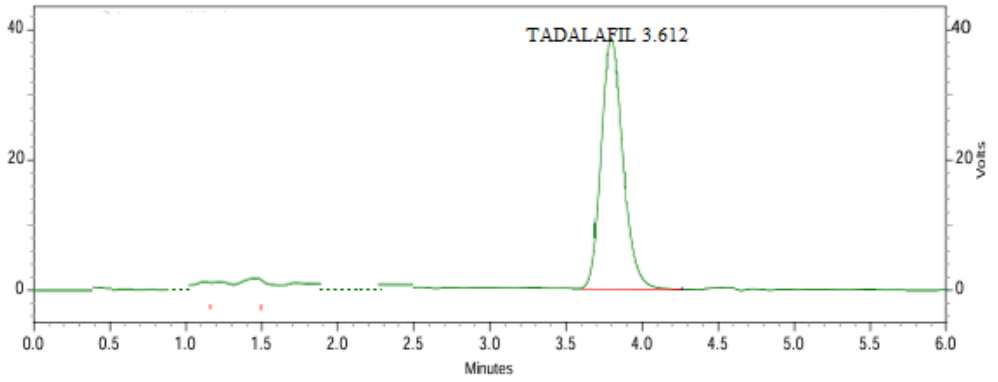


Fig. 8: Chromatogram for Tadalafil at Thermal degradation.

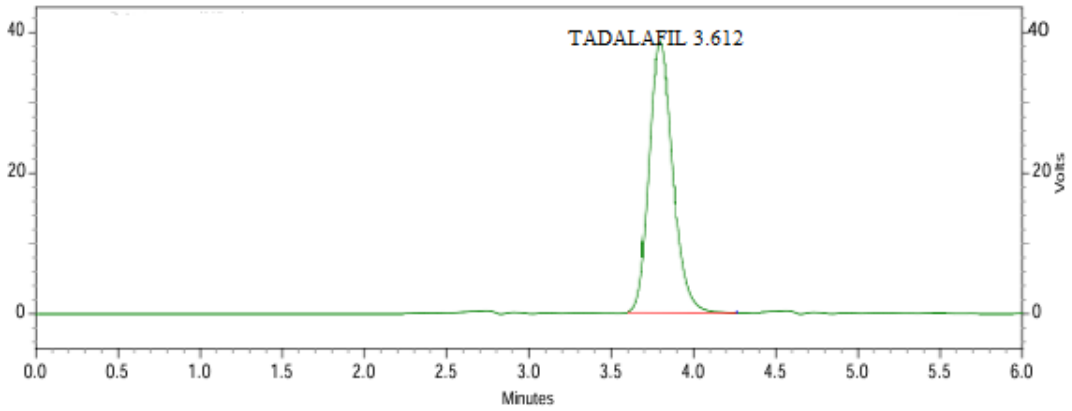


Fig. 9: Chromatogram for Tadalafil at Photolysis degradation.

Table 9: Stress degradation behavior of Tadalafil.

Stress condition	% Drug recovered	% Drug decomposed	Tailing factor	Theoretical Plates	Peak Purity
Standard drug	100		1.21	5170	100
Acidic Hydrolysis (0.1N HCl, 30 min at 60°C)	94.8	4.1	1.32	5145	100
Alkaline Hydrolysis (0.1N NaOH_30 min at 60°C)	81.5	16.5	1.28	5180	100
Oxidative degradation (3% H2O2 30 min at 60°C)	95.6	3.8	1.17	5178	100
Thermal degradation (80°C, 12 hrs.)	98.60	1.3	1.21	5489	100
Photolytic degradation (24 hrs.)	99.4	0.7	1.31	5789	100

This study's methodology is simple, sensitive, accurate, robust, durable, quick, and exact. Common tablet excipients do not obstruct detection, as demonstrated by the chromatographic analysis's lack of extra peaks. For the accurate measurement of tadalafil in tablet formulations, the approved RP-HPLC method is appropriate.

CONCLUSION

Tadalafil can be determined using the developed stability-indicating approach, which is selective and does not reveal influence from excipients or degradation products. Tadalafil in tablet dosage forms can be routinely analyzed using this approach.

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