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Research Article

"Development And Validation Of An Rp-Hplc Method For Tadalafil Quantification Utilizing A Quality By Design (Qbd) Strategy"

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ABSTRACT:
A fast, straightforward, correct, and strong RP-HPLC analytical procedure based on a The Quality by Design (QbD) methodology, was developed for the examination of tadalafil in both its API and Finished Formulations. The Effective separation was achieved by chromatography by means of an Reversed-phase C18 column (X Bridge) with constant Carrier fluid composition."The chromatographic resolution employed a Carrier fluid of phosphate buffer (pH 6.8) and acetonitrile in a 60:40 (v/v) proportion." "Analysis was conducted with a photodiode array (PDA) detector at a wavelength of 284 nm, operating at a flow rate of 1.0 mL/min.".
In order to maximize the chromatographic circumstances, a Design of Experiments (DoE) strategy was applied Under the QbD methodology. The experimental design focused on two main factors: (i) independent variables, including the run rate and the proportion of acetonitrile in the Carrier fluid , and (ii) co-variables such as elution time, theoretical plates, and the tailing factor. Statistical analysis of the design was performed using techniques such as ANOVA, normal residual plots, Box-Cox plots for power transformation, perturbation plots, 3D response surface plots and contour plots. The technique was authenticated in accord with ICH-Q2B guidelines. "These include parameters such as linearity, limits of detection and quantification, accuracy, precision, specificity, and robustness.". The findings demonstrated the technique's straightforwardness, high sensitivity, and dependability, confirming its strong suitability for the routine evaluation of tadalafil.

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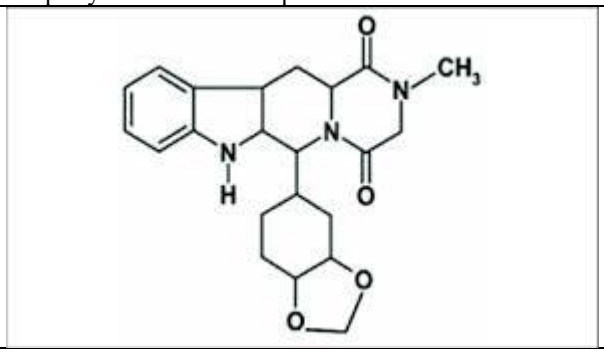
INTRODUCTION

The crystalline powder of tadalafil is white to off-white in color. It dissolves somewhat in methanol and somewhat in water. The molecular mass of tadalafil is 438.4 g/mol, and its Structural formula is C22H19N3O4. After oral treatment, tadalafil is quickly absorbed. It takes 30 to 120 minutes to attain the maximal plasma concentration. The cytochrome P450 enzyme system breaks down tadalafil in the liver. 17.5 hours is the elimination half-life. One PDE5 inhibitor is tadalafil. One of the enzymes that breaks down cGMP is PDE5. One chemical that is involved in erectile function is called cGMP. Tadalafil aids in boosting cGMP levels in the penis, which causes an erection, by preventing the breakdown of cGMP.

Drug Name	Tadalafil
Brand Name	Cialis
Class	inhibitor of phosphodiesterase type 5 (PDE5)
Indications	Benign prostatic hyperplasia (BPH) and erectile dysfunction
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, flushing, and upset stomach. Seek medicinal consideration if you skill any of these symptoms.
Storing	Keep dry and at room temperature.
Structure	
IUPAC	(2R,8R)-2-(1,3-benzodioxol-5-yl)-6,6-dimethyl-3,6,17-triazatetracyclo[8.7.0.0^{3},^{8}.0^{11},^{16}]heptadeca-1(10),11,13,15-tetraene-4,7-dione

METHODS

MATERIALS AND METHODS

Chemicals and solvents:

Merck Lifesciences Pvt. Ltd., Mumbai, India produces HPLC grade methanol (LichrosolR), and HPLC water 2487 MPOWER 2. Sigma-Aldrich (USA) kindly provided the working standards for Tadalafil and Cialis tablets were purchase from local market.

INSTRUMENTATION:

The study employed a UV spectrophotometer featuring a Photodiode Array (PDA) detector, specifically the Alliance 2996 model, to determine the λ max values of the pharmaceutical compounds. For method development, a non-endcapped ZORBAX C18 column (250 x 4.6 mm, 5 µm) was used. Chromatographic analysis was managed using Empower analytical software. Ultraviolet (UV) finding was passed out at a wavelength of 284 nm. An isocratic elution technique was employed, utilizing a Carrier fluid containing of phosphate buffer (pH 6.8) and acetonitrile in a 60:40 volume proportion. The run speed was continued at 1 mL per minute. The separation process was carried out with a 20 µl injection volume, and finding was again at 284 nm. All measurements were accompanied at a temperature of 25°C.

Stock and standard solutions.

Tadalafil was made into a primary "A stock solution was prepared at a concentration of 1 milligram per milliliter." using the carrier fluid as the solvent.. The same carrier fluid was then used to dilute this stock, creating a working standard solution with a 50 µg/mL concentration. Through additional dilution, a number of standard solutions were created from this working solution, yielding concentrations 10 µg/mL to 50 µg/mL is the range.

Sample preparation.

To create a consistent powder, the tablets of Cialis were pulverized into a fine powder.. A piece of this powder that had been precisely weighed and equal to 100 mg of Tadalafil was put into a 100 ml volumetric flask with 50 ml of the carrier fluid. A 1 mg/ml concentration in a stock solution was produced by this procedure. To guarantee adequate dissolution, the mixture was sonicated for ten minutes. Subsequently, extra carrier fluid was added to get the volume down to 100 ml. Whatman Grade 1 filter paper (110 mm in diameter) was used to filter the resultant solution, which was then put away in an appropriate container for further examination. The assay sample was made by passing the stock solution through a 0.22 µm membrane filter after diluting it with the carrier fluid to a 50 µg/ml as the final concentration.

Method development and experimental design.

To accomplish successful chromatographic separation, "RP-HPLC, which stands for reverse-phase high-performance liquid chromatography"was a method that recently created. It uses a mobile phase made up of phosphate buffer (pH 6.8) and acetonitrile in a volume ratio of 60:40. To evaluate there silience A

Box-Behnken Design (BBD) was used for statistical analysis and experimental planning in this analytical approach. Because BBD is efficient and requires fewer experimental runs when numerous variables (three or four) are included, it was chosen over a central composite design.

Design-Expert® application was used to process the investigational data (version 10.0.3.1). The percentage of acetonitrile in the carrier fluid (factor A, % v/v) and the flow rate (factor B, mL/min) were the main independent variables examined in this study. The tailing factor (R2), Residence time (RT, R1, min), and theoretical plate count (TP, R3, N) are the three dependent variables were used to assess the method's performance.A two-factor, three-level Box-Behnken Proposal was used for all nine trials. Table 1 provides comprehensive details about the levels of the independent and dependent variables. Through statistical tests like ANOVA and model fit evaluation, the design's dependability was validated. Additionally, the chromatographic conditions were optimized and fine-tuned using response surface methodology (RSM).

Method validation

Tadalafil was measured using a novel analytical method that was Authenticated in compliance with the (ICH) direction.The suitability of the system was confirmed by six consecutive injections of a standard solution with a concentration of 50 µg/mL and an injection volume of 20 µL each. The relative standard deviation (%RSD) of the maximum responses and the average tailing factor were assessed in order to assess the method's consistency.

Linearity.

A standard stock solution of tadalafil was subjected to serial dilution to prepare samples with concentrations of 10, 20, 30, 40, and 50 µg/mL. To assess the sensitivity of the analytical method, the limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively..

Precision.

The accuracy of the new method was evaluated by examining both repeatability (intra-assay precision) and intermediate precision. To assess repeatability, six replicate injections of 20 µl were performed using a standard solution with a concentration of 50 µg/ml. For intermediate precision, the same procedure six replicate injections of 20 µl at 50 µg/ml was repeated across six consecutive days. Precision was quantified by calculating the relative standard deviation (RSD) percentage of the results obtained.

Accuracy.

To test the correctness of the settled technique and determine how formulation additives affected the analysis, the sample solution was fortified with a reference medication material at concentrations of 80%, 90%, 100%, 110%, and 120%. Every measurement was passed out in **Specificity.**

To determine specificity, three separate injections of the Tadalafil blank, standard, and sample solutions were performed. The peak purity analysis confirmed the results.

RESULTS AND DISCUSSION

Based on the ideas of Quality by Design (QbD), a factorial design technique was used to improve the suggested methodology. Assessing the effects of two important independent variables was the aim of the design: the acetonitrile concentration and flow in the mobile phase (referred to as

rate, which is frequently referred to as factor B. The three chromatographic response parameters that were assessed in this study were the number of theoretical plates (R3), tailing factor (R2), and retention length (R1). The chosen variable ranges and fixed chromatographic parameters are shown in Table

Table No. 1: Independent variable selection and level

variable	names	unit	Type	Coded value			Actual value		
				low	mid	high	low	mid	high
A	ACN	%	Numeric	-1	0	1	25	30	35
B	Flow Rate	ml/min	Numeric	-1	0	1	0.8	0.9	1

Table no 2-Box behanken experimental design using variables and their responces

standarad	run	A:% of ACN	B: flow rate ml/min	Retention time (min)	Tailing factor	Theorotical plate
8	1	0	-1	2.90	1.362	5110
2	2	0	-1	3.40	1.375	5212
5	3	0	0	2.85	1.36	5109
6	4	1	0	2.90	1.44	5450
7	5	-1	1	3.50	1.19	5070
4	6	-1	0	4.1	1.29	5065
9	7	1	1	2.50	1.41	5270
3	8	1	-1	3.20	1.43	5640
1	9	-1	-1	4.5	1.291	5250

Table no 3 ANOVA result for response R1 (RT)

Source	Sum of Square	df	Mean square	F value	P value	significant
model	2.78	2	1.38	23.20	0.0014	significant
A % ACN	1.90	1	1.90	31.08	0.0012	
B-Flow Rate	0.86	1	088	14.65	0.0084	
Residual	0.35	6	0.058			
Cor Total	3.12	8				

Table no 4 ANOVA result for response R 2 (RT)

Source	Sum of Square	df	Mean square	F value	P value	significant
model	0.031	2	0.014	386.58	0.0001	significant
A % ACN	0.031	1	0.031	764.36	0.0001	
B-Flow Rate	3.839 E-004	1	3.839E-004	9.80	0.0203	
Residual	2.348E-004	6	2.348E-005			
Cor Total	0.032	8				

Table no 5 ANOVA result for response R 3 (RT)

Source	Sum of Square	df	Mean square	F value	P value	significant
model	2.280E+004	2	1.141E+004	9.23	0.0147	significant
A % ACN	1.568E+004	1	1.564E+004	12.70	0.0116	
B-Flow Rate	71284.00	1	71285.00	5.74	0.0428	
Residual	73906.33	6	2.348E-005			
Cor Total	3.019E+004	8				

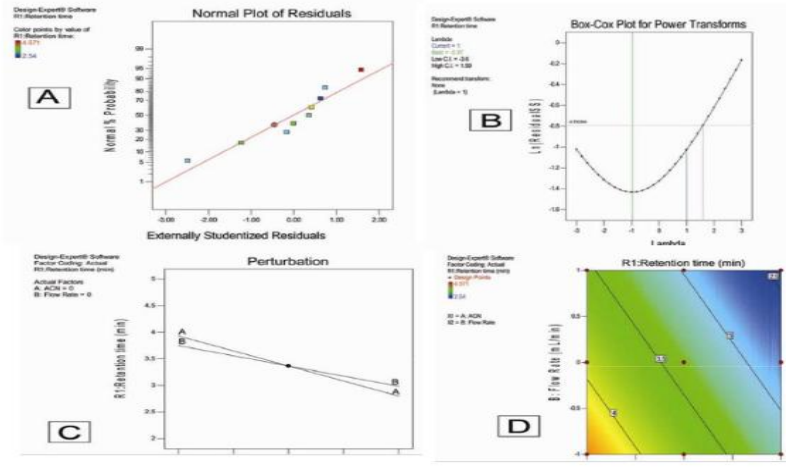
Table no 6 Summary statistics for response R1,R2,R3

Response	R1(RT)	R2(TF)	R3(TP)
Std dev	0.23	6.255 E-003	6.255E-003
Mean	3.36	1.35	1.35
C.V %	7.24	0.44	0.44
Press	0.67	5.832E-004	5.832E-004
R-Squared	0.8859	0.9922	0.5752
Adjusted R- Squared	0.8433	0.9891	0.6741
Predicted R-Squared	0.7820	0.9807	0.4746
Adequate Precision	13.42	43.54	8.41

Table no 7 The optimized method according to design of experiments

Method	**% of ACN**	**Flow Rate Ml/Min**	**RT min**	**TF**	**TP**	**Desirability**
Predicted Level	0.642	0.849				
Actual value	33.22	0.983	2.788	1.314	5290.50	**1**
Experimental	32.99	1	2.752	1.324	5301.12	
Deviation			0.910	0.58	0.142	

Qbd Method for RP-HPLC Development and Validation



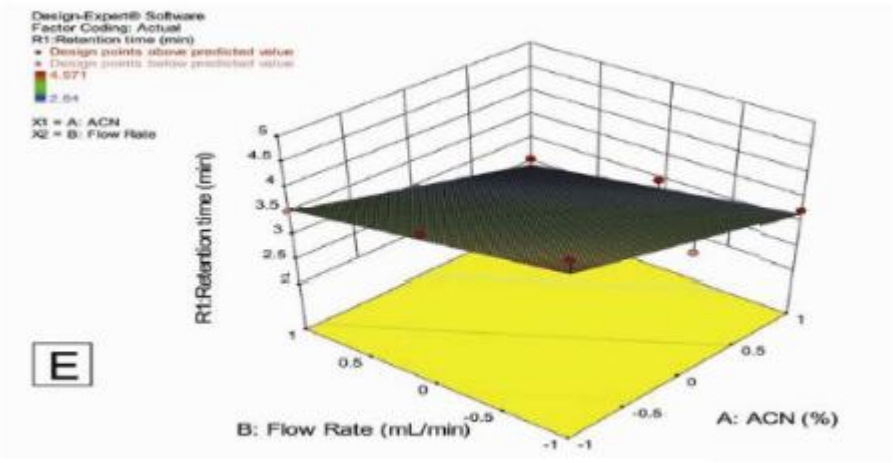
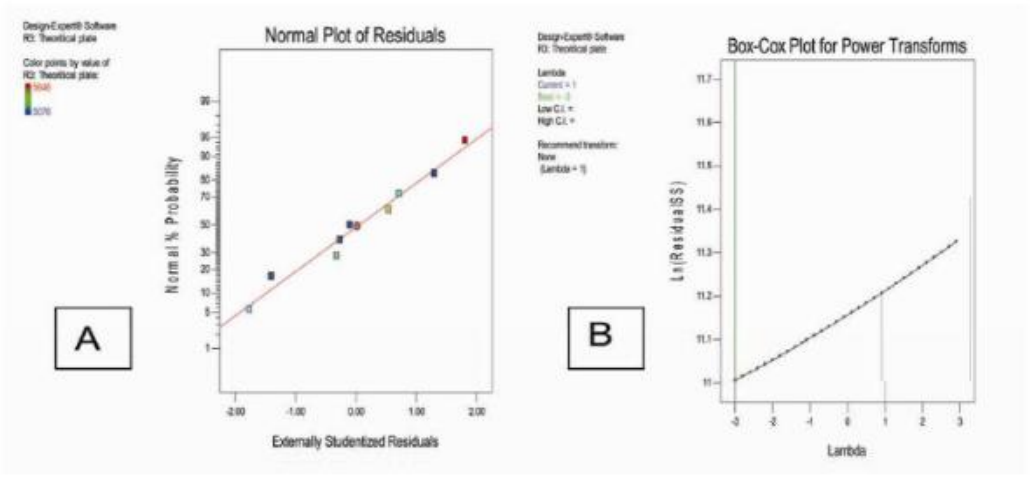
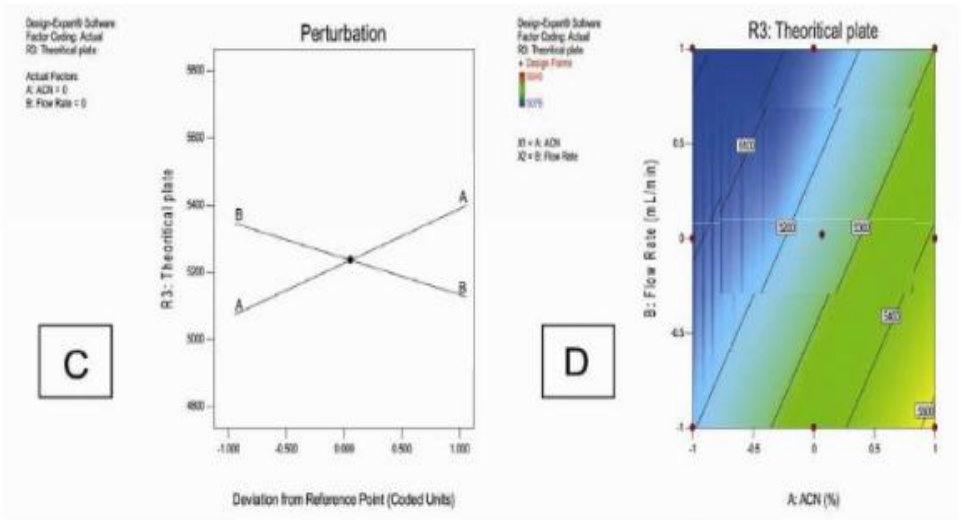


Figure 2. (A)- Normal probability plot of residuals, (B)- Box-Cox plot for determining the optimal power transformation,, (C)- Perturbation plot showing the influence of variables on the response, (D)- Contour plot illustrating the interaction effects on R1,, (E)- 3D surface plot demonstrating the combined impact of factors on R1.





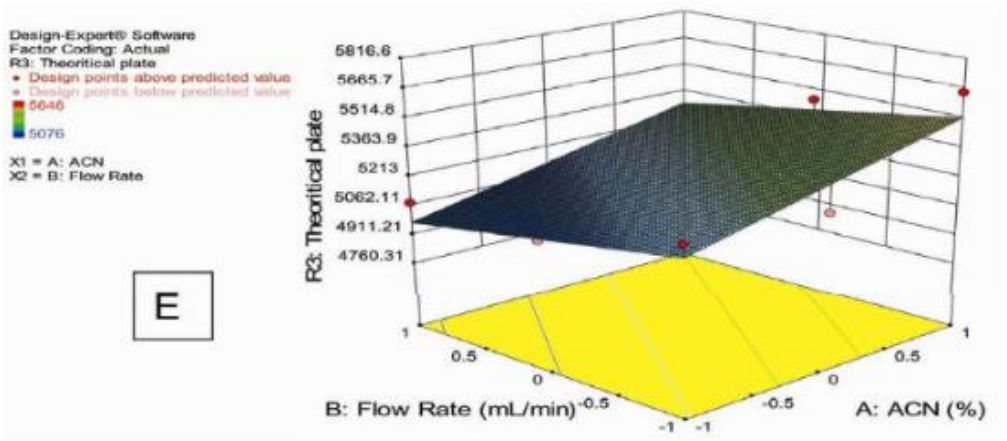


Figure 3. (A) Normal probability plot of residuals, (B) Box-Cox transformation plot for identifying optimal power transformation, (C) Perturbation plot showing sensitivity of factors, (D) Contour plot illustrating factor interactions, (E) Three-dimensional response surface depicting the influence on R3

To assess the predictive capability of the developed model, nine experimental trials were conducted under controlled conditions. The combinations of Carrier fluid composition and flow rate used in these trials were based on the Box-Behnken Design (BBD), as detailed in Table 2. Data on theoretical plate count (TP), retention time (RT), and tailing factor (TF) were gathered throughout each trial. A randomized order was used for the experimental runs in order to lessen the impact of uncontrollable outside factors.

Among the models evaluated, the quadratic model demonstrated superior predictive performance, as evidenced by the highest coefficients of determination for the three response variables (R1, R2, and R3). A Lack of Fit test was carried out to verify the adequacy of the model, and results indicated that the lack of fit was statistically insignificant, with the p-value exceeding the model's F-value. Residual analysis, including normal probability plots, confirmed the absence of significant outliers, as data points closely followed the reference line (see Figures 2, 3, and 4-A). Analysis of Variance (ANOVA) was used for additional validation, and the results showed that the model was statistically significant. Strong match was shown by the F-values of 23.20, 386.58, and 9.23 for R1, R2, and R3, respectively. In each of the three answers, both factors A and B

In Tables 3, 4, and 5, B were statistically significant ($p < 0.05$).

The adjusted R-squared values, presented in Table 6, were 0.8433 for R1, 0.9891 for R2, and 0.6741 for R3. These values were nearby to the expected R-squared values—0.7820, 0.9807, and 0.746, respectively—with differences of less than 0.2, suggesting good agreement between the observed and predicted responses. Additionally, the signal-to-noise ratios for all responses were well above the acceptable threshold of 4.0, with values of 13.42, 43.54, and 8.41, indicating reliable model performance.

The model serves as a useful tool for exploring and optimizing the experimental design space. The quadratic equations and three-dimensional response surface plots illustrate how both factors A and B influence the outcomes. Specifically, both variables negatively impact retention time (Figure 2), while their effects on tailing factor (Figure 3) and theoretical plate count (Figure 4) are mixed. This confirms the non-linear nature of the relationships, where simultaneous changes in the input variables can lead to complex response patterns.

Overall, the statistical analysis provides insight into the dependencies between the response variables RT, TF, and TP, enabling better control and optimization in experimental conditions.

Table no 8 Accuracy of Tadalafil

Spike level %	% Recovered ± %RSD
80	98.50 ± 0.02
90	99.50.10 ± 0.04
100	99.70 ± 0.14
110	99.30 ± 0.07
120	100.40 ± 0.04

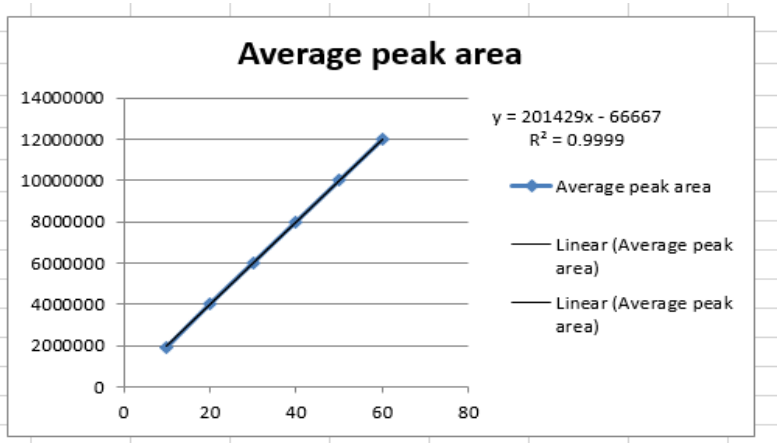
Table 9. Inter-day and intra-day precision summary for Tadalafil.

Inter -day	Time (hr)	0	1	2	4	8	12
	Assay %	98.55	98.15	98.40	98.90	100.10	101.1
	Mean ± SD			99.52 ± 0.969			
	% RSD			0.969			
	Day	1	2	3	4	5	6

Intra-day	Assay %	99.99	99.50	99.45	99.10	98.80	98.60
	Mean ± SD			99.10 ± 0.471			
	% RSD			0.479			

Table 10 Linearity data for Tadalafil.

Sr.no	Concentration(micro gram/ml)	Average peak area
1	10	1900000
2	20	4000000
3	30	6000000
4	40	8000000
5	50	10000000
6	60	12000000



Fi 4-Linearity curve for Tadalafil.

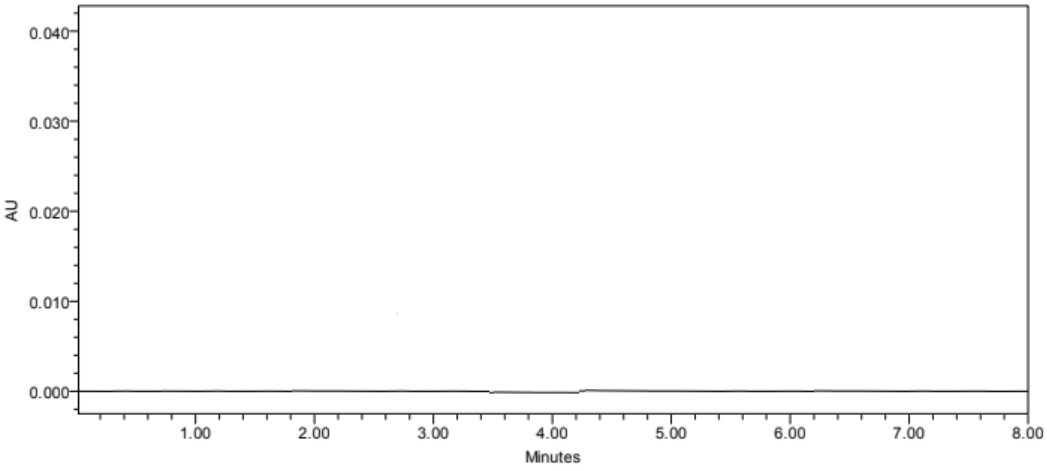


Fig 5-chromatogram for blank

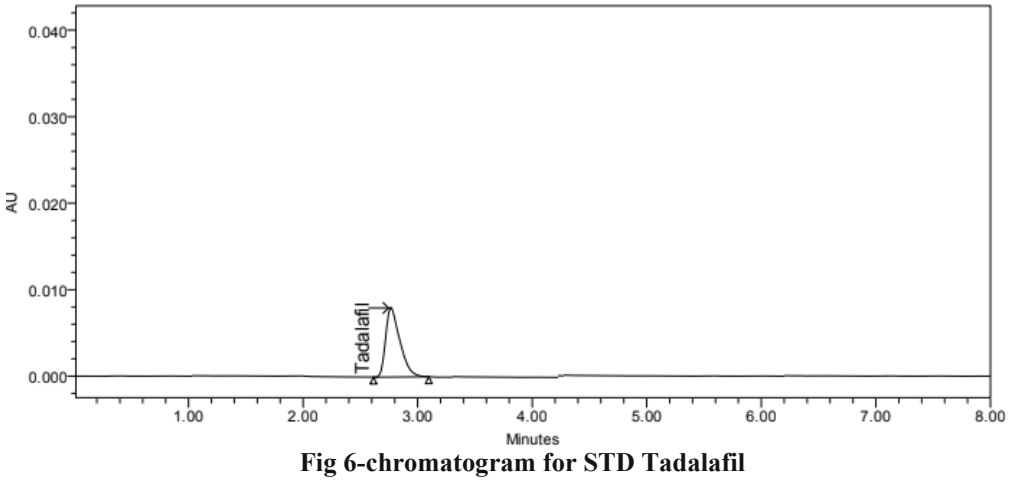


Fig 6-chromatogram for STD Tadalafil

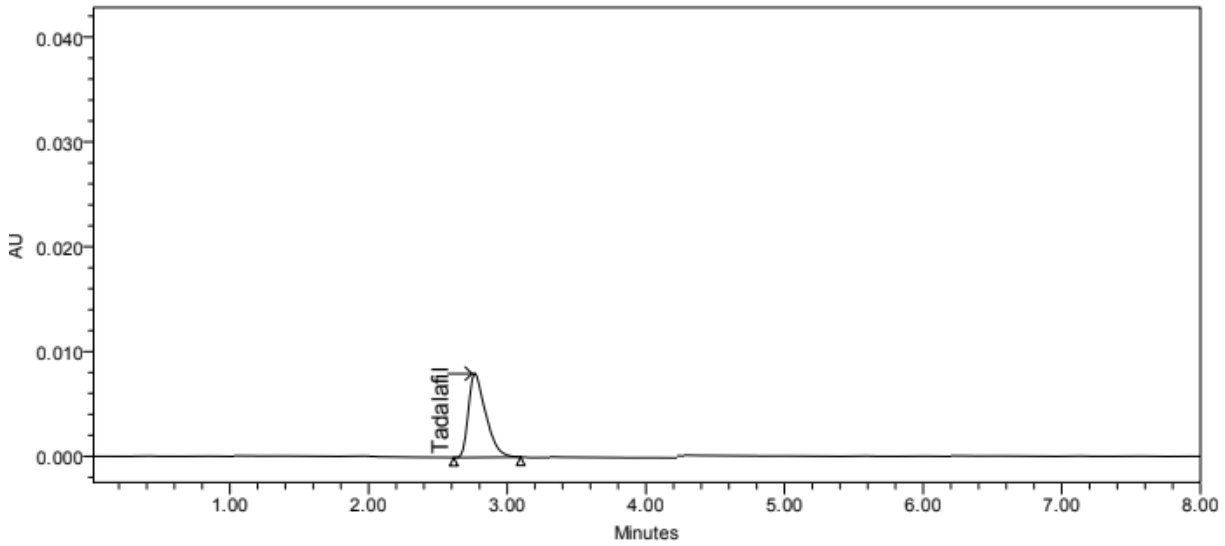


Fig 6-chromatogram for SAM Tadalafil

Variations in the experimental settings had no discernible effect on the quantitative analysis of tadalafil, demonstrating the analytical method's resilience. Every outcome fell within the permissible range of no more than 2.0%, and the experimental results of the suggested

The results showed that the approach was in respectable agreement with the suggested values.

Validation of the method

A high coefficient of determination (R2 = 0.9984) suggests that there is a strong linear association between Tadalafil concentrations and the relevant HPLC peak regions. The regression equation, Y = 201429X - 66667, was followed by the calibration curve, where X

represents the concentration of tadalafil (µg/mL), and Y is the peak area that was observed (see Figure 4). With LOQ of 0.05 µg/mL and a LOD of 0.01 µg/mL, the devised analytical technique showed exceptional sensitivity.Accuracy and precision were verified by recovery testing, and (RSD) of below 2% supported the technique's consistency (see Table 9).

Findings for intermediate precision and repeatability both satisfied accepted criteria, maintaining RSD readings less than the 2% threshold. Additionally, chromatographic examination showed that the Tadalafil peak was well-resolved and that neither the standard nor the sample chromatograms showed any observable interference from excipients or other components (Figures 6 and 7).

Conclusion

A reverse-phase high-performance liquid chromatography (RP HPLC) approach was created utilizing the Quality by Design (QbD) methodology in order to examine tadalafil.It has been demonstrated that the approach is linear, accurate, exact, repeatable, and specific. Additionally, it provides excellent sensitivity, simplicity of usage, and outstanding selectivity, as demonstrated by its low detection limit and distinct retention time. It is a solid contender for regular quality control in pharmaceutical applications because of these qualities.

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