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CONCURRENT QUANTIFICATION OF TADALAFIL AND SILDENAFIL IN COMBINED TABLET FORMULATION USING RP-HPLC METHOD

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Abstract
The current work aims to establish a validated RP-HPLC method for estimating the combined dosage of sildenafil and tadalafil. Using ZORBAX C18 (250 × 4.6 mm, 5 µm)as a stationary phase and mobile phase, the medicines were separated The mobile phase consisted of acetate buffer pH 3.0 adjusted with acetic acid and methanol (10:90, v/v) at a flow rate of 0.7 mL/min., at 280 mm detection was carried out. Sildenafil's Rt was 4.611 minutes, while Tadalafil's was 3.744 minutes. Tadalafil and sildenafil had respective percentage drug contents of 98.63% when the commercial formulation was examined using the established approach. In the 50–150 PPM range for Tadalafil and 50–150 PPM range for Sildenafil, the approach was determined to be linear.. The results showed that the quantitation limit for sildenafil was 1.65 PPM and the detection limit for tadalafil was 5 PPM. For both medications, the accuracy and precision scores were found to be close to 100% w/w. Additionally, the approach was proven to be specific and robust. Tadalafil and sildenafil in combination dosage form were found to be linear, specific, sensitive, precise, accurate, and robust when analyzed using the proposed RP-HPLC technique.

Keyword: Tadalafil and Sildenafil, HPLC, Validation

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

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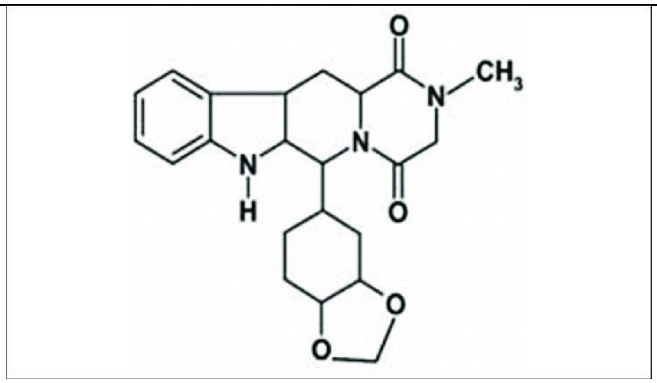
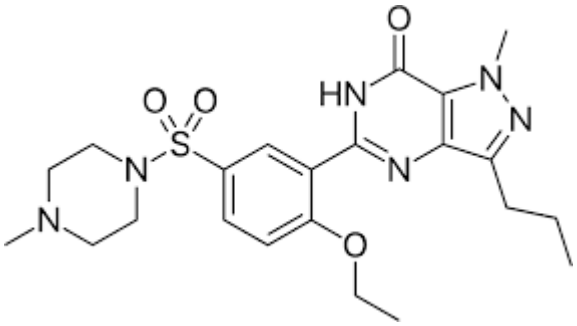
Structure	
IUPAC	(6R,12Ar)-6-(1,3-Benzodioxol-5yl)-2-methy1-2,3,6,7,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]bindole-1,4-dione.

Sildenafil is a crystalline powder that ranges from White of- white. It is soluable in methanol and just weakly soluable in water. Sildenafil's molecular Wight is 474.6 g/mol and its chemical formula is C22H30N6O4S. When sildenafil is taken orally, it is quickly absorbed. Within 30 to 120 minutes, the plasma concentration reaches its maximum. The cytochrome P450 enzyme system breaks down sildenafil in the liver. Three to five hours is the elimination half-life. One PDE5 inhibitor is sildenafil. One enzyme that degrades cGMP is PDE5. One signaling molecule involved in erectile function is cGMP. Sildenafil helps to raise the amounts of cGMP in the penis, which results in an erection, by preventing the breakdown of cGMP.

Table 2: Drug profile for Sildenafil

Drug Name	**Sildenafil**
Brand Name	Viagra
Class	Phosphodiesterase type 5 (PDE5) inhibitor
Indications	Erectile dysfunction, benign prostatic hyperplasia (BPH)
Dosage	25-100 mg orally 30-60 minutes before sexual activity
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 encompass cephalalgia, facial erythema, and gastrointestinal distress; it is imperative to pursue medical evaluation should any of these manifestations occur.
Storage	Store in dry palace at room temperature
Structure	
IUPAC	5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-d]pyrimidin-7-one;2-hydroxypropane-1,2,3-tricarboxylic acid

MATERIALS AND METHODS

Chemicals and solvents:

HPLC grade methanol (Lichrosolv**,** Merck Life sciences Pvt. Ltd., Mumbai, India), HPLC water 2487 MPOWER 2. Acetate buffer, composed of sodium acetate, acetic acid, and distilled water, was employed in the investigation. The

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analytical standards for Tadalafil and Sildenafil were graciously provided as a donation from Sigma-Aldrich (USA). The Sildalist tablet, which comprises 100 mg of Sildenafil and 20 mg of Tadalafil was acquired from the local commercial market, produced by Torrent Pharmaceutical Company.

Chromatographic Conditions

A ZORBAX C18 (250 mm × 4.6 mm, 5 µm) column with acetate buffer (pH 3.0) and methanol (10:90, v/v) as the mobile phase under isocratic mode at a flow rate of 0.7 mL/min was used to perform chromatographic separation. With a run time of eight minutes, detection was carried out at 280 nm. Before being used, the mobile phase was degassed and passed through a 0.45 µm membrane filter.

Preparation of stock solution, working standard solution. Mobile phase and buffer solution.

Preparation of Acetate Buffer

The required quantity of sodium acetate was weighed and dissolved in distilled water. The buffer system was made by adjusting its pH using acetic acid. It was then passed through a membrane filter having a pore size of 0.45 µm and sonicated.

Preparation of Mobile Phase

Acetate buffer with methanol (ratio 10:90) has been prepared. Acetate buffer with pH value of 3.0 has been made by adding acetic acid, and then two solvents have been combined and filtered using 0.45µm membrane filter. The two solvents were degassed and prepared for chromatographic analysis following the equilibration of the first two solvents.

Preparation of standard solution

Two stock solutions, Sildenafil and Tadalafil, were prepared in a concentration of 200 ppm by dissolving 20.0 mg of each drug in 100 mL of Acetate Buffer/Methanol 10:90 (v/v), an acetate buffer solution at a pH of 3.0 using acetic acid to adjust the pH value. Both stock solutions were then diluted from their original concentrations to provide working standards for linearity evaluations, between 50 and 150 ppm.

Preparation of Sample Solutions of sildenafil and Tadalafil

Weigh 20 Tablets, average, and then finely grind them. Add the equivalent of 20 mg of Sildenafil and 20 mg of Tadalafil to a 100 mL volumetric flask; dissolve the two in acetate buffer: methanol (10:90 v/v), pH 3.0 (adjusted to pH 3.0 with acetic acid); sonicate for 15 minutes. After sonication, dilute to desired final volume; this will provide both drugs with a stock solution of 200 ppm. Filter stock solution through a 0.45 µm membrane filter; dilute further with mobile phase to obtain final analytical concentration.

Preparation of Calibration curve

A series of diluted solutions covering 50–150 ppm were prepared from the primary stock of each analyte. The prepared solutions were analyzed under established chromatographic conditions, and the detector responses were recorded. A calibration plot was generated by correlating concentration with peak response, followed by regression analysis to determine the linear relationship.

Determination of Wavelength

Sildenafil and Tadalafil standard solutions were scanned in the UV area to determine their corresponding maximum absorbences (λ max). The overlapped spectra were then evaluated to find an appropriate common λmax for both analytes that will provide sufficient absorbance for use in chromatographic methods.

Determination of Formulation

The prepared sample solution of the marketed tablet formulation was analyzed under optimized chromatographic conditions. The peak areas obtained for Sildenafil and Tadalafil were compared with those of corresponding standard solutions. The drug content was calculated using the respective calibration curves and expressed as percentage of labeled claim.

Method Validation

The RP-HPLC method was developed and validated to the standards of the ICH Q2(R1) guidelines. The following validation parameters were analyzed: specificity, linearity, accuracy, precision (repeatability and intermediate precision), robustness, limit of detection, limit of quantification, solution stability, and system suitability

Specificity.

The specificity of the analytical method was assessed by separately analyzing blank, standard, and sample preparations under established chromatographic conditions. The obtained chromatograms were checked for the presence of interfering peaks at the retention times of Sildenafil and Tadalafil. Since no interference was detected, the method was considered selective and specific for the estimation of both drugs.

Linearity.

The linearity of the assay was assessed by using a standard solution and evaluating results for the concentration range of 50 - 150 PPM. The calibration curve for each analyte was built through regression analysis of the peak area versus the respective concentration and then determining the correlation coefficient.

Accuracy.

The accuracy of the analytical method was assessed using the standard addition technique at three concentration levels of 50%, 100%, and 150%. The recoveries obtained at each level were determined, and the corresponding percentage relative standard deviation (%RSD) values were calculated to confirm the accuracy of the developed method.

Precision.

Repeatability was evaluated through numerous injections of a standard solution, whereas intermediate precision was studied

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over several days. Results were presented as the percentage relative standard deviation of peak area and retention time.

Robustness.

The robustness was assessed by introducing minor intentional alterations in chromatographic condition, including flow rate and detection wavelength .The effect of these variations on system suitability parameters was examined. The method was found to be robust as no significant changes in chromatographic performance were observed.

LOD and LOQ.

The Limit of Detection and Limit of Quantification were determined using the standard deviation of the response and the slope of the calibration curve in accordance with ICH guidelines.

Solution Stability.

Test and standard solutions were stored in ambient conditions and inspected at predetermined intervals. The stability of the solutions was assessed by comparing the obtained chromatographic responses and calculating the percentage relative standard deviation.

Dissolution Study.

Dissolution analysis was conducted using USP Apparatus II fitted with a paddle stirrer operating at 50 rpm. The study employed 900 mL of dissolution medium maintained at 37 ± 0.5°C during the entire experiment. Samples were withdrawn at selected time points, clarified through filtration, and quantified using the validated chromatographic analytical procedure.

Assay of marketed formulation.

A powder was made from twenty measured Tablets. One tablet's worth of material was dissolved in diluent, transferred to a 100 mL volumetric flask, and sonicated for 25 minutes. After filtering the solution, 1 mL of the filtrate was diluted to 10 mL using the same diluent to obtain the analytical solution.

Method Development And Validation.

Method Development.

A reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed in order concomitantly determine Tadalafil and sildenafil, with the aim of achieving good separation, satisfactory peak symmetry and short run time.'Different mobile phase systems were explored in optimization of the method. The first trials with phosphate buffer-acetonitrile did not give sufficient resolution, and acetate buffer-methanol did not give sufficient resolution but gave longer retention and broader peaks. Optimization of acetate buffer:methanol (10:90 v/v) at pH 3.0 gave sharp and symmetrical peaks with good resolution and reduced retention time, which is an indicator of a good chromatographic performance.

Table 3. Optimization Trials of Chromatographic Conditions for RP-HPLC Method Development of Tadalafil and Sildenafil.

Trial No.	Column Specification	Mobile Phase Composition (% v/v)	Observation / Remark
1	ZORBAX C18 (250 × 4.6 mm, 5 µm)	20 mM KH_2PO_4 buffer : Acetonitrile (50:50), pH 3.5 (adjusted with OPA)	Inadequate separation of analytes with poor resolution
2	ZORBAX C18 (250 × 4.6 mm, 5 µm)	Acetate buffer : Methanol (50:50), pH 3.0 (adjusted with acetic acid)	Improved peak shape; however, prolonged retention time and peak broadening observed
3	ZORBAX C18 (250 × 4.6 mm, 5 µm)	Acetate buffer : Methanol (70:30), pH 3.0 (adjusted with acetic acid)	Excessively long retention time with unsatisfactory separation
4	ZORBAX C18 (250 × 4.6 mm, 5 µm)	Acetate buffer : Methanol (10:90), pH 3.0 (adjusted with acetic acid)	Well-defined, sharp, and symmetrical peaks with satisfactory resolution and reduced retention time

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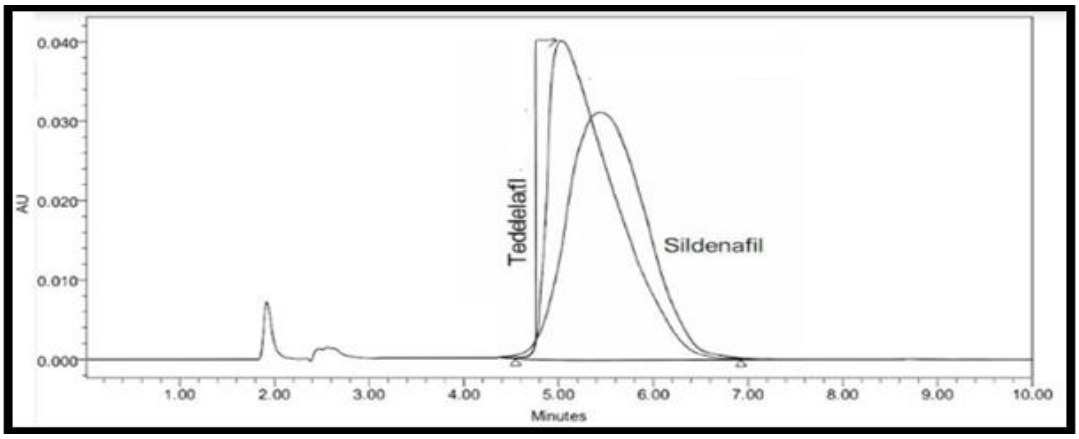

Figure 1. Trial no 1 '(20 mM KH_2PO_4 buffer: acetonitrile (50:50, v/v), pH 3.5 (OPA)'

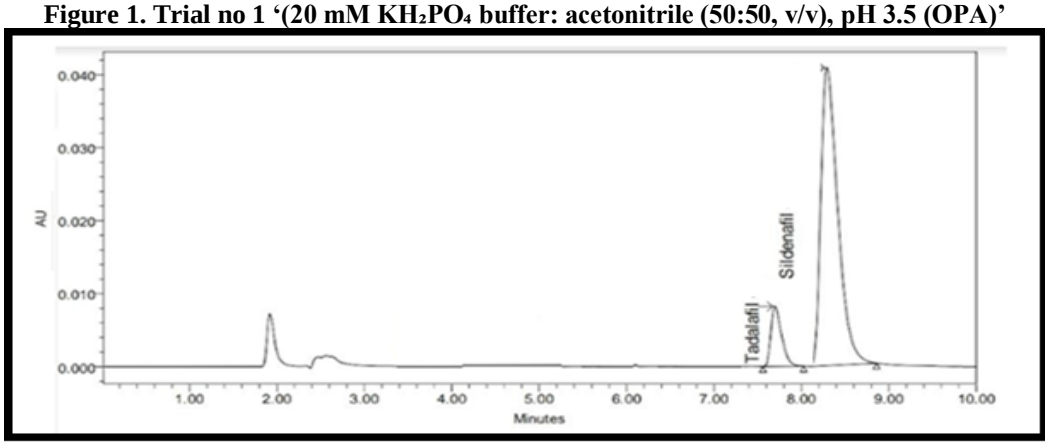

Figure 2. Trial no 2 '(Acetate Buffer: Methanol 50:50) pH 3.0 adjusted with acetic acid'

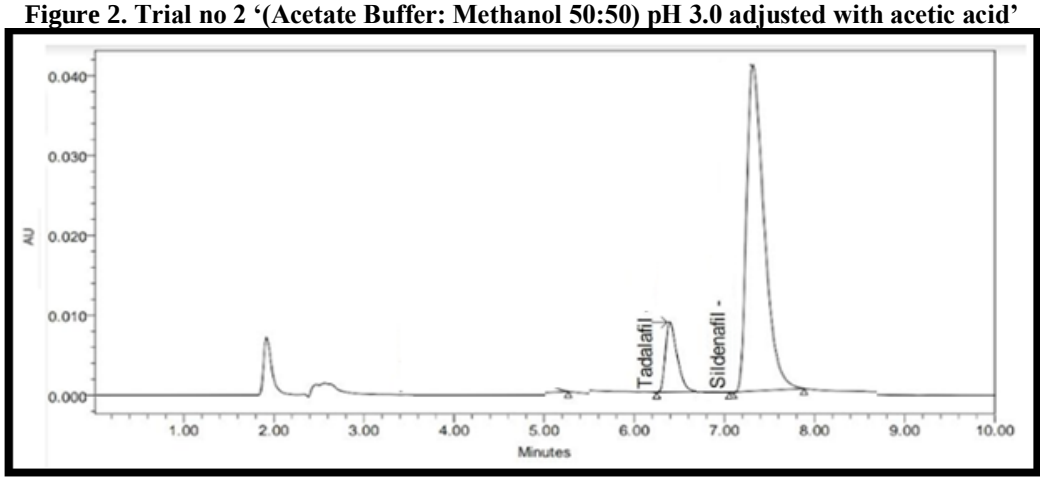

Figure 3. Trial no 3 '(Acetate Buffer: Methanol 70:30) pH 3.0 adjusted with acetic acid'

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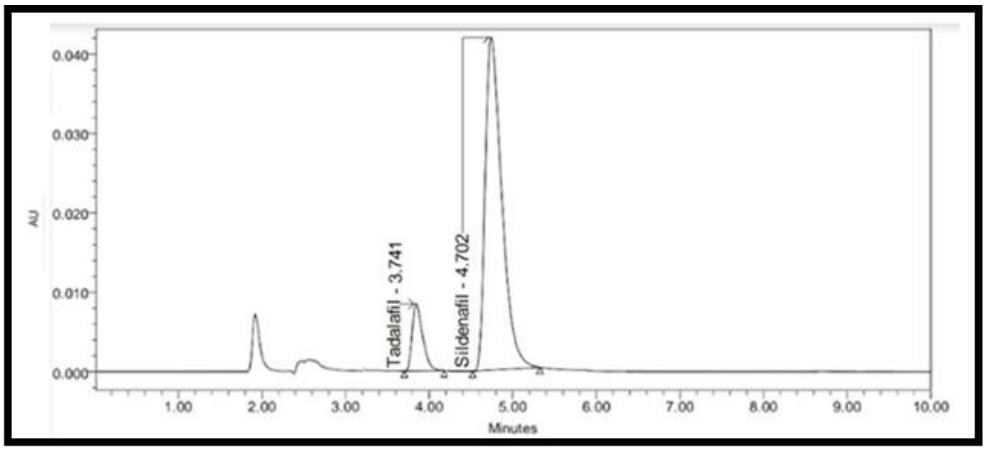
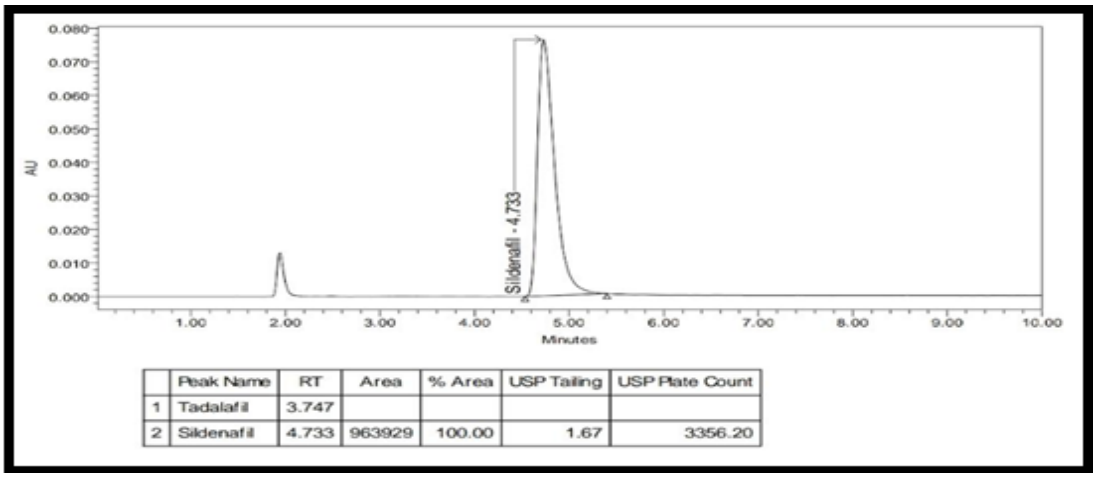


Figure 4. Trial no 4 (Acetate Buffer: Methanol 10:90) pH 3.0 adjusted with acetic acid

Optimized Chromatographic Conditions.

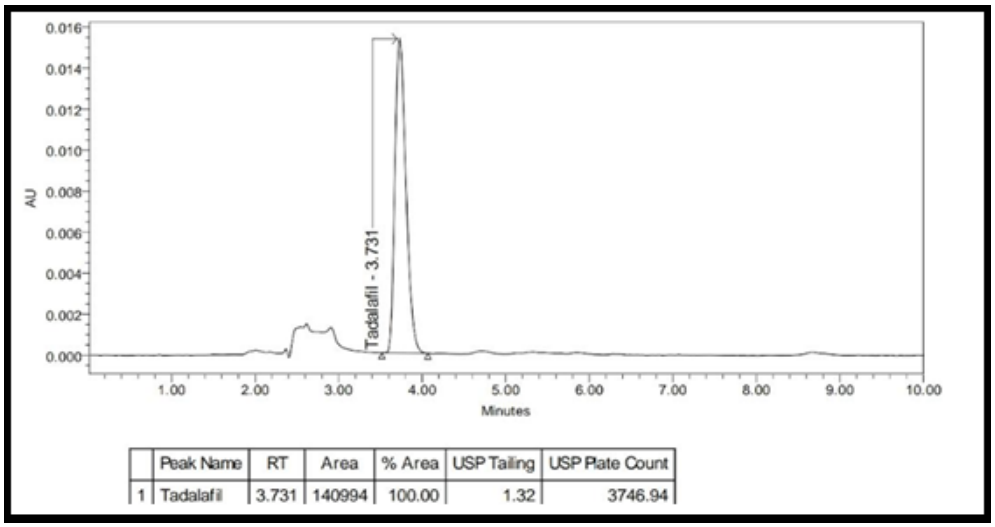
System Suitability.

To ensure correct chromatographic functioning, system suitability was checked before analysis. The retention time of Sildenafil and Tadalafil was 4.71 min and 3.74 min respectively and the resolution was 2.85 which is satisfactory. The values of tailing factors were less than 2.0 and theoretical plate counts exceeded 2000 showing good plate symmetry and column efficiency. The precision was good with a %RSD of less than 2.0.Even though there were slight changes in the number of plates, they did not influence the overall performance. The system was considered to be suitable to be analyzed.



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Tadalafil	3.747				
2	Sildenafil	4.733	963929	100.00	1.67	3356.20

Figure 5. Normal chromatogram for Sildenafil STD



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Tadalafil	3.731	140994	100.00	1.32	3746.94

Figure 6.Normal chromatogram for Tadalafil STD

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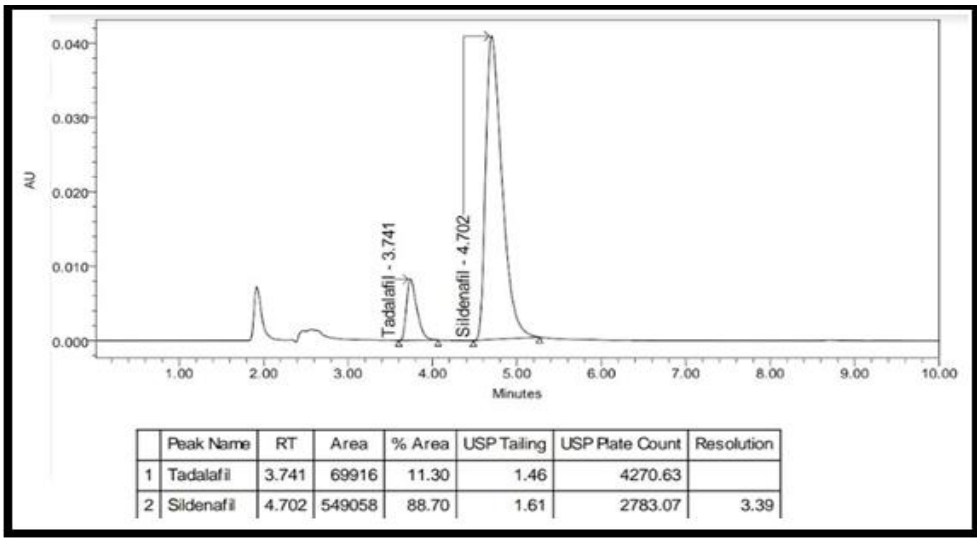
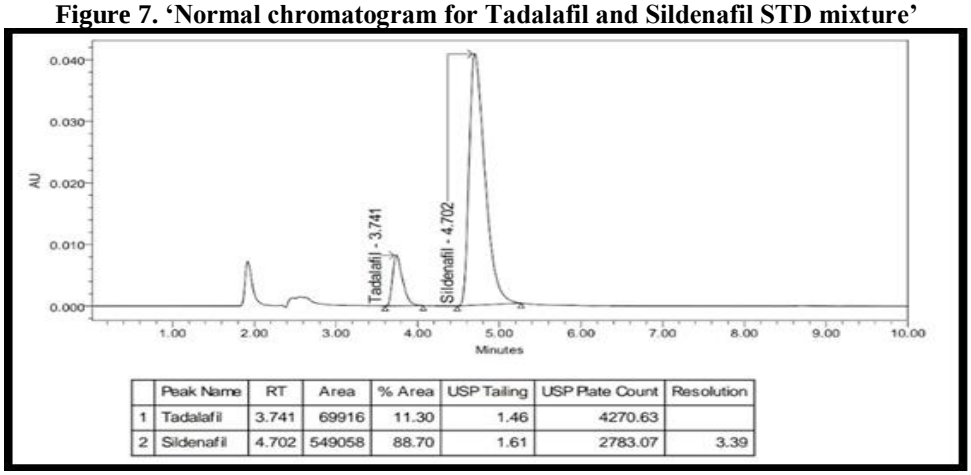


	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.741	69916	11.30	1.46	4270.63	
2	Sildenafil	4.702	549058	88.70	1.61	2783.07	3.39

Figure 7. 'Normal chromatogram for Tadalafil and Sildenafil STD mixture'



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.741	69916	11.30	1.46	4270.63	
2	Sildenafil	4.702	549058	88.70	1.61	2783.07	3.39

Figure 8. 'Normal chromatogram for Tadalafil and Sildenafil sample
6.2.1.4 Method Validation.'

Method Validation

Linearity

The analyses showed a linear response in 50-150 ppm concentration of both analyses using RP-HPLC technique.Tadalafil showed a regression equation of $y = 751.4x - 1921.6$ ($R^2 = 0.9993$), while Sildenafil showed $y = 5280.6x - 9267.2$ ($R^2 = 0.9996$). The correlation coefficients are also large and it is an indication that there is a powerful linear relationship that the method is acceptable to be employed in the quantitative analysis in this range.

Table 4.Linearity for Tadalafil

S. No.	Concentration (ppm)	Area
1	50	35176
2	80	57932
3	100	74280
4	120	88656
5	150	110049

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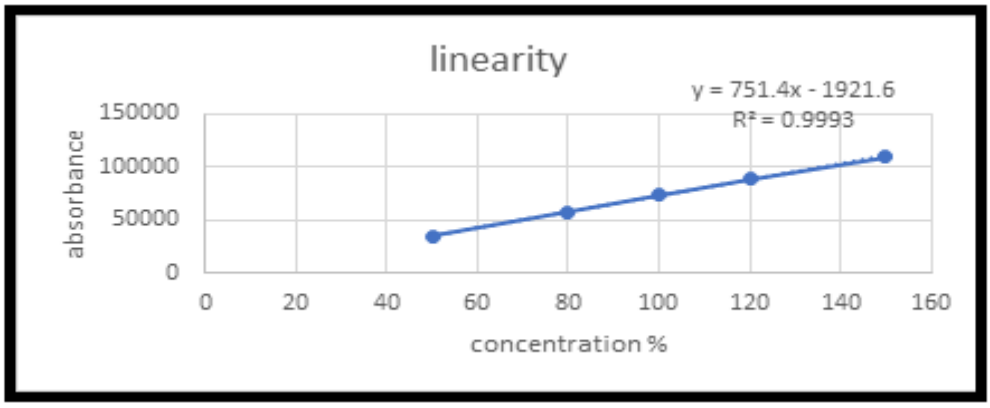

Figure 9. Linearity for Tadalafil

Table 5. Linearity for Sildenafil

Sr. No.	Concentration (ppm)	Absorbance (Area)
1	50	258322
2	80	407259
3	100	517853
4	120	627931
5	150	782604

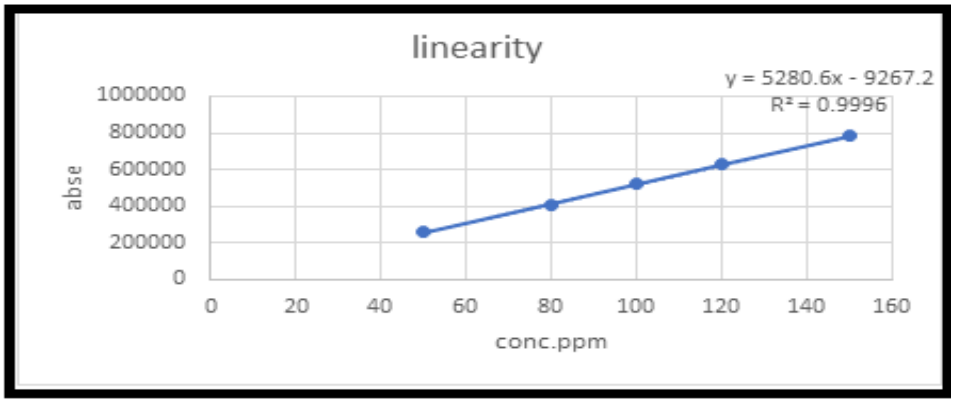

Figure 10. Linearity for Sildenafil

Accuracy.

Precision of the procedure was tested using standard addition at 50, 100 and 150 percent of the nominal assay level. Recoveries of Sildenafil and Tadalafil were 99.65 to 100.45 with lower values of the percent RSD than 1.0. This implies that these results represent acceptable trueness and accuracy, which is within ICH Q2(R1) specifications.

Table 6. Accuracy (Recovery Study) for Tadalafil and Sildenafil

Level	Drug	Mean % Recovery	SD	%RSD
50%	Tadalafil	99.72	0.32	0.32
50%	Sildenafil	99.65	0.41	0.41
100%	Tadalafil	100.12	0.28	0.28
100%	Sildenafil	100.08	0.36	0.36
150%	Tadalafil	100.45	0.30	0.30
150%	Sildenafil	99.84	0.38	0.38

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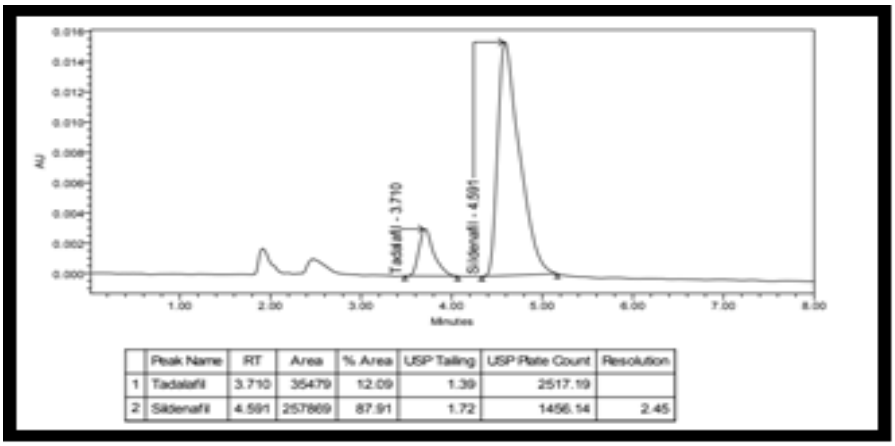

Figure 11. 'Chromatogram for Tadalafil and Sildenafil Accuracy 50%'

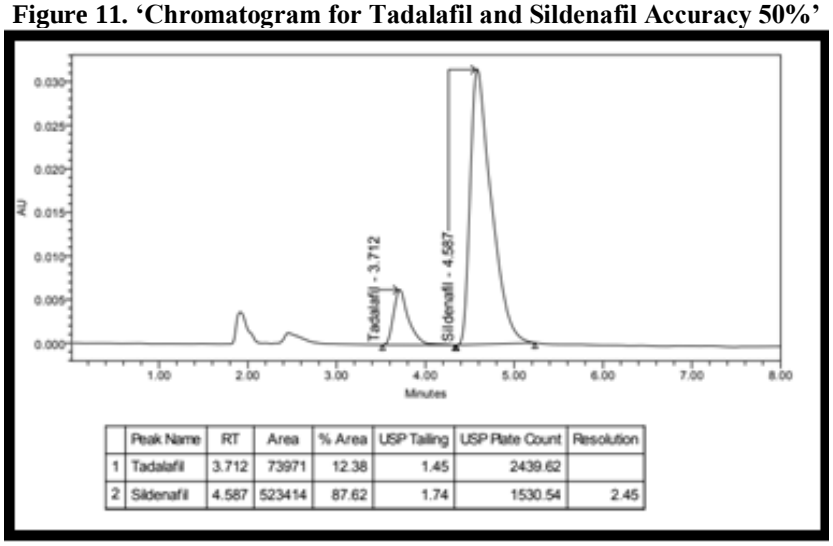

Figure 12. 'Chromatogram for Tadalafil and Sildenafil Accuracy 100 %'

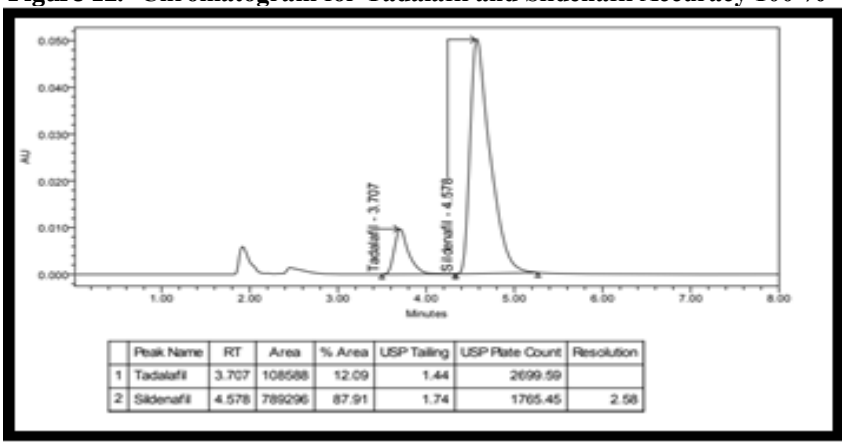

Figure 13. 'Chromatogram for Tadalafil and Sildenafil Accuracy 150%'

High flow variation

The robustness of the method was determined by varying the flow rate of 0.7 to 0.8 mL/min with other chromatographic conditions kept constant. There was a slight decrease in the retention time without influencing the symmetry and efficiency of the peak. Both analytes had their %RSD of peak area of less than 2% with acceptable tailing factor, plate count (>2000), and resolution (>2.0). These results affirm that the approach is resilient to the change of flow rate.'

Table 7. High Flow Variation Data Sildenafil (n = 5)

Injection No.	Retention Time (min)	Peak Area	% Area	USP Tailing	USP Plate Count
1	4.266	500539	88.85	1.67	2603.91
2	4.272	501786	88.77	1.70	2589.91
3	4.267	503425	88.79	1.69	2555.72

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4	4.271	501535	88.67	1.69	2598.47
5	4.279	501794	88.76	1.71	2480.66

Table 8. Statistical Summary

Parameter	Mean	SD	%RSD
Retention Time (min)	4.271	0.005	0.12
Peak Area	501815.8	1036.84	0.21
% Area	88.77	0.07	0.08
USP Tailing	1.69	0.015	0.89
USP Plate Count	2565.73	47.86	1.86

Table 9. High flow variation Data for Tadalafil

Injection No.	Retention Time (min)	Peak Area	% Area	USP Tailing	USP Plate Count
1	3.398	62783	11.15	1.51	4371.51
2	3.408	63531	11.24	1.62	4160.89
3	3.405	64091	11.33	1.61	4269.37
4	3.397	63527	11.21	1.58	4199.26
5	3.403	63458	11.23	1.59	4209.03

Table 10.Statistical Summary

Parameter	Mean	SD	%RSD
Retention Time (min)	3.402	0.004	0.11
Peak Area	63478.0	465.02	0.73
% Area	11.23	0.07	0.62
USP Tailing	1.58	0.04	2.53
USP Plate Count	4202.01	76.33	1.82

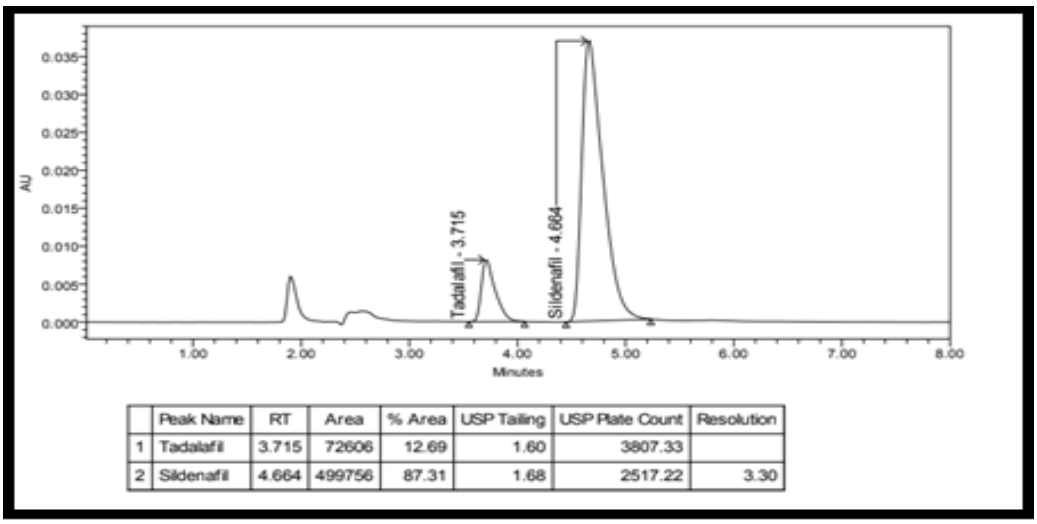

Figure 14. Chromatogram for High flow variation

LOD & LOQ.

The limits of detection (LOD) and quantification (LOQ) were used to establish the sensitivity of the developed RP-HPLC method in line with ICH Q2(R1) guidelines.The lowest analyte concentration that can be reliably determined under the stated experimental conditions, but not necessarily determined with a reasonable degree of accuracy, is called the LOD. Conversely, when the LOQ is low, this indicates the highest level of concentration that can be quantitatively measured with the appropriate level of accuracy and precision, thus the assay method is reliable at low concentration levels.. LOD and LOQ

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values were calculated using the standard deviation of intercept and slope of the calibration curve in accordance with ICH Q2(R2). Experimental verification confirmed signal-to-noise ratio >3 for LOD and >10 **for** LOQ.'

Table 11. LOD and LOQ Results for Sildenafil and Tadalafil

Parameter	**Sildenafil**	**Tadalafil**
LOD (ppm)	1.65 ppm	1.65 ppm
LOQ (ppm)	5.0 ppm	5.0 ppm
Retention Time at LOD (min)	4.611	3.744
Retention Time at LOQ (min)	4.614	3.752
S/N Ratio at LOD	>3	>3
S/N Ratio at LOQ	>10	>10
Tailing Factor at LOQ	1.37	1.16
Plate Count at LOQ	3959	4997

The designed RP-HPLC procedure had a high sensitivity and LOD of 1.65 ppm and LOQ of 5 ppm of both compounds. The RSD at LOQ level was less than 2% which is reliable.Therefore, the method is sufficiently sensitive for routine quantitative analysis

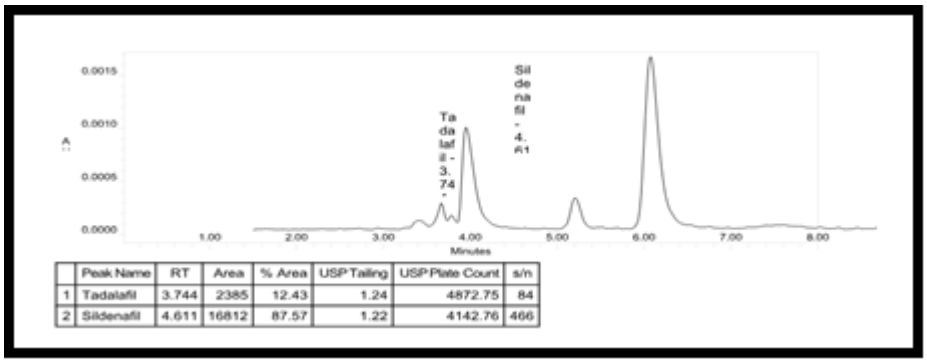

Figure 15. 'Normal chromatogram LOD & LOQ for STD'

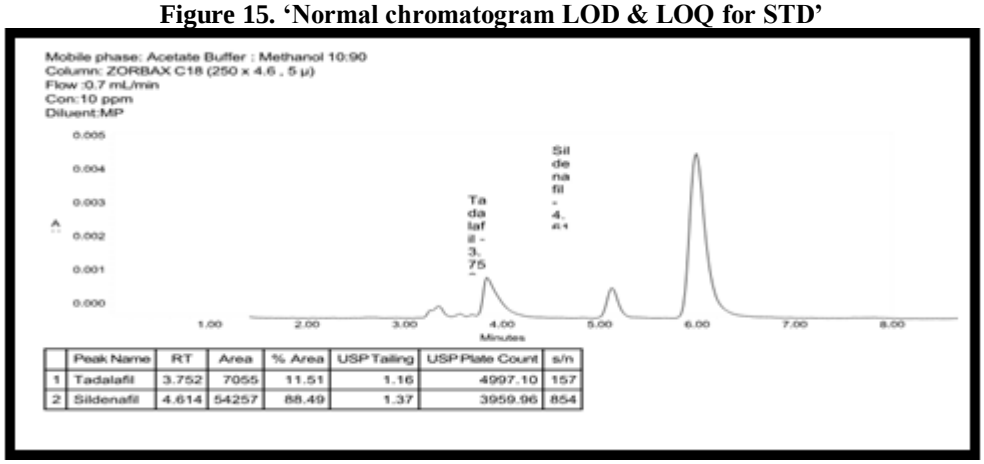

Figure 16. 'Normal chromatogram LOD & LOQ for SAM'

Intermediate Precision:

Intermediate precision was evaluated by performing analyses on different days and runs within the same laboratory. The value of the RSD was 1.11% and 1.58% in Sildenafil and Tadalafil respectively, and the retention time was varying by less than 1%. These findings suggest a high reproducibility and prove the validity of the method in the case of intermediate precision.

Table 12. 'Intermediate Precision Results for Sildenafil (n = 12)'

Parameter	**Mean**	**Standard Deviation**	**%RSD**
Retention Time (min)	4.636	0.022	0.47
Peak Area	569681.7	6318.3	1.11
% Area	88.78	0.18	0.20

Table 6.26. 'Intermediate Precision Results for Tadalafil (n = 12)'

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Parameter	Mean	Standard Deviation	%RSD
Retention Time (min)	3.694	0.016	0.44
Peak Area	71958.2	1137.8	1.58
% Area	11.23	0.16	1.42

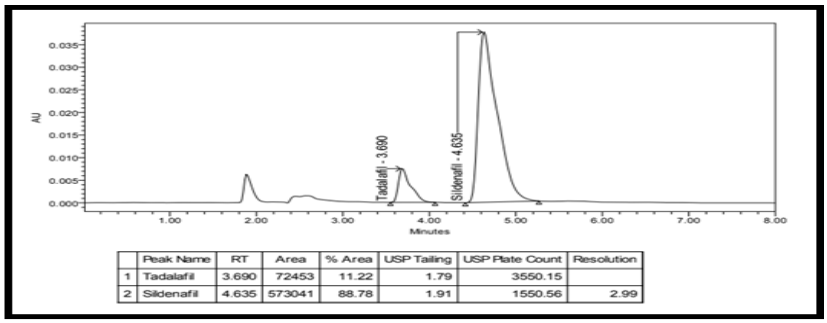

Figure 17. Normal chromatogram Intermediate Precision

Solution stability.

Solution stability was confirmed by repeated analysis of standard and test preparations under identical chromatographic conditions. The %RSD of peak area was 0.77% for Sildenafil and 0.84% for Tadalafil, with retention time variation below 1%, indicating consistent performance. These findings show that both analytes were stable throughout the study and that the procedure is a good way of quantifying them.

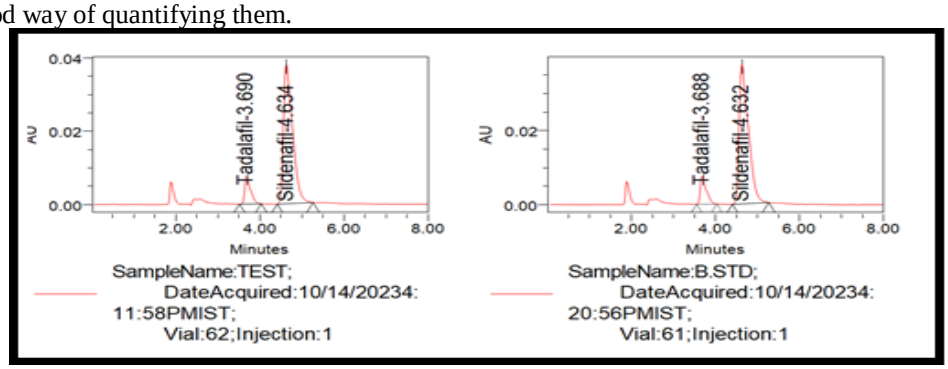

Figure 18. 'Normal chromatogram Solution stability'

Table 13. 'Solution Stability Data Sildenafil'

Sr. No.	Sample Name	Vial	Inj.	Analyte	Retention Time (min)	Area	% Area	USP Tailing	USP Plate Count
1	Blank	60	1	Sildenafil	4.707	—	—	—	—
2	B.STD	61	1	Sildenafil	4.632	572142	88.86	1.86	1525.77
3	STD	61	1	Sildenafil	4.632	574563	88.83	1.85	1678.53
4	STD	61	1	Sildenafil	4.626	571258	88.78	1.87	1594.16
5	STD	61	1	Sildenafil	4.630	569867	88.79	1.82	1734.33
6	STD	61	1	Sildenafil	4.627	574885	88.87	1.88	1646.35
7	STD	61	1	Sildenafil	4.627	574106	88.83	1.89	1713.38
8	Test	62	1	Sildenafil	4.634	562212	88.85	1.83	1812.68

Table 14. Statistical Summary (Excluding Blank)

Parameter	Mean	Standard Deviation	%RSD
Retention Time (min)	4.639	0.028	0.59
Peak Area	571290.5	4410.9	0.77

Though slight reduction in theoretical plate count was observed during solution stability studies, chromatographic performance remained acceptable

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Table 15. Solution stability of Tadalafil

Sr. No.	Sample Name	Vial	Inj.	Analyte	Retention Time (min)	Area	% Area	USP Tailing	USP Plate Count
1	Blank	60	1	Tadalafil	3.747	—	—	—	—
2	B.STD	61	1	Tadalafil	3.688	71761	11.14	1.72	3224.31
3	STD	61	1	Tadalafil	3.687	72227	11.17	1.75	2120.06
4	STD	61	1	Tadalafil	3.686	72020	11.13	1.72	2407.98
5	STD	61	1	Tadalafil	3.688	71939	11.21	1.70	2698.13
6	STD	61	1	Tadalafil	3.686	72174	11.22	1.74	2847.57
7	STD	61	1	Tadalafil	3.689	72246	11.17	1.73	2206.02
8	Test	62	1	Tadalafil	3.690	70529	11.15	1.70	3121.31

Table 16.Statistical Summary (Excluding Blank)

Parameter	Mean	Standard Deviation	%RSD
Retention Time (min)	3.695	0.021	0.57
Peak Area	71842.4	604.7	0.84

Wavelength variation.

Strength with regard to wavelength of detection was tested by changing the wavelength by 2 nm, as well as, 280 nm.. The least variation was found at 278 nm and 282 nm in Sildenafil and Tadalafil with percent relative standard deviation of less than 2 and retention time variations of less than 1%. These findings prove that the procedure is stable and consistent even in the case of tiny wavelength differences.

Table 17. Robustness Study Effect of Wavelength Variation on Tadalafil (n=5)

Wavelength (nm)	Parameter	Mean	Standard Deviation	%RSD
278 nm	Peak Area	72210.6	158.4	0.22
	Retention Time (min)	3.688	0.002	0.05
282 nm	Peak Area	72024.2	175.6	0.24
	Retention Time (min)	3.687	0.001	0.04

Table 18. Robustness Study Effect of Wavelength Variation on Sildenafil (n=5)

Wavelength (nm)	Parameter	Mean	Standard Deviation	%RSD
278 nm	Peak Area	631953	1041.2	0.16
	Retention Time (min)	4.684	0.001	0.03
282 nm	Peak Area	499117	539.2	0.11
	Retention Time (min)	4.668	0.003	0.06

Dissolution study.

The evaluation of dissolution samples of Sildenafil and Tadalafil was done using the developed RP-HPLC method. Dissolution testing: USP Apparatus II (paddle method) was used, and 900 mL of the dissolution medium (37 ± 0.5 °C) was used, with a paddle rotation rate of 50 rpm. At the designated time, samples were collected and examined with the help of the designed RP-HPLC technique.

Table 19.Dissolution Study Results for Sildenafil (n = 3)

Injection No.	Standard Peak Area	Dissolution Sample Peak Area
1	572142	598734
2	574563	595812
3	571258	597421
Mean	572654	597322

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SD	1658.24	1474.63
%RSD	0.29%	0.25%

Table 20.Dissolution Study Results for Tadalafil (n = 3)

Injection No.	Standard Peak Area	Dissolution Sample Peak Area
1	71761	74892
2	72227	74436
3	72020	74618
Mean	72003	74649
SD	233.6	229.8
%RSD	0.32%	0.30%

Percentage Drug Release Formula.

$$\%Drug\ Release = \frac{Sample\ Peak\ Area}{Standard\ Peak\ Area} \times \frac{Standard\ Concentration}{Sample\ Concentration} \times 100$$

Table 21. Percentage Drug Release:

Parameter	Sildenafil (% Drug Release)	Tadalafil (% Drug Release)
Unit 1	99.12	100.21
Unit 2	98.87	99.76
Unit 3	99.45	100.08
Mean	99.15	100.02
SD	0.29	0.23
%RSD	0.29%	0.23%

The dissolution study of Sildenafil and Tadalafil showed low variability in peak areas (%RSD < 1%), confirming good system precision.Mean drug release was 99.15% for Sildenafil and 100.02% for Tadalafil, with %RSD below 0.5%, indicating uniform and consistent release. All results meet pharmacopeial limits (NLT 80%), demonstrating that the method is precise and suitable for routine quality control analysis.

Results of assay of marketed formulation

Individual weight of tablets was measured and the mean weight of the tablet was estimated. A 100 mL volumetric flask was weighed and the appropriate amount of one tablet loaded into it. About 50 mL of diluent was put in and the mixture was sonicated within the next 25minutes to be able to extract the drug contents fully. The solution that resulted was filtered to eliminate insoluble excipients. Then, 1 mL of the filtrate was poured into a 10 mL volumetric flask and diluted to volume in the same diluent to get the final analytical solution.

Assay Formula

$$\%Assay = \frac{A_T}{A_S} \times \frac{C_S}{C_T} \times 100$$

Table 22. Assay of marketed formulation

Brand Name	Drug	Label Claim (mg/tablet)	Standard Peak Area	Sample Peak Area	% Assay	%RSD
Sildalist	Sildenafil	100 mg	799456	789296	99.73%	0.91
Sildalist	Tadalafil	20 mg	108690	108588	100.45%	1.21

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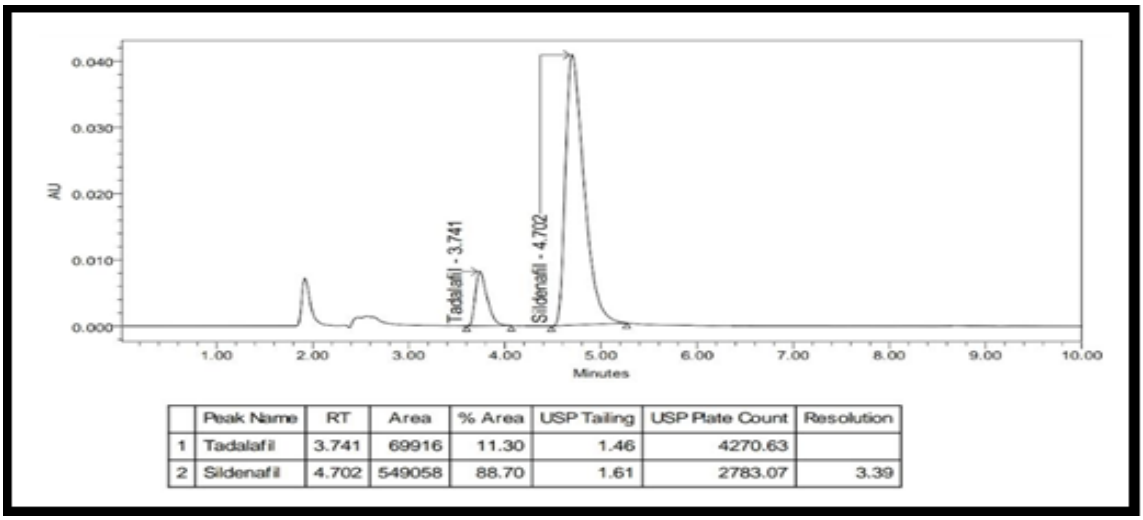

	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.741	69916	11.30	1.46	4270.63	
2	Sildenafil	4.702	549058	88.70	1.61	2783.07	3.39

Figure 19. Normal chromatogram for Tadalafil and Sildenafil Tablet

Overall Conclusion

A robust, precise, and sensitive RP-HPLC method was successfully developed and validated for the simultaneous estimation of Sildenafil and Tadalafil in bulk drug and tablet dosage form. The method complied with ICH Q2(R2) requirements for specificity, linearity, accuracy, precision, robustness, sensitivity, and solution stability. The assay and dissolution results were within acceptable limits, demonstrating the suitability of the method for routine quality control and pharmaceutical analysis.

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