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HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF TADALAFIL AND SILDENAFIL IN BULK AND TABLET DOSAGE FORM

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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 x 4.6, 5 µ) as a stationary phase and mobile phase, the medicines were separated appropriately: Acetate Buffer: Methanol 10:90 with a mobile phase 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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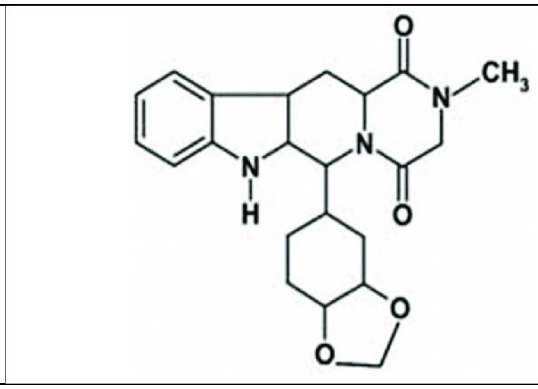
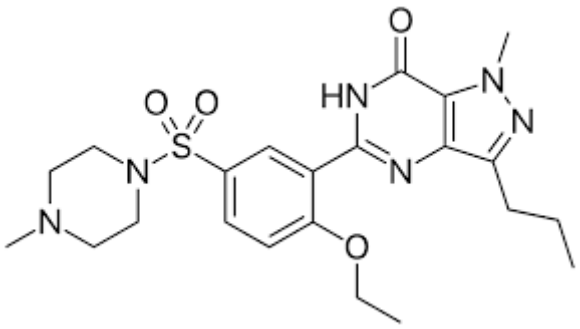
Structure		
IUPAC	(6R,12Ar)-6-(1,3-Benzodioxol-5yl)-2-methy1-2,3,6,7,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]bindole-1,4-dione.	

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 (LichrosolR , 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 analytical standards for Tadalafil and Sildenafil were graciously provided as a donation from Sigma-Aldrich (USA). The

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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.

Instrumentation

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 utilized for the purpose of method development. The chromatographic system was operated under the control of Empower analytical software. The analytes were scrutinized through UV detection at a wavelength of 280 nm, utilizing an isocratic mode with the mobile phase consisting of Acetate Buffer and Methanol in a 10:90 ratio; the flow rate was set at 0.7 ml/min. In alignment with the specifications of the flow chamber, the concentration rate was consistently preserved at 1.0 ml/min, while UV absorbance measurements were recorded at 280 nm. The operational temperature of the oven and the duration of the analytical run were regulated at 37°C and 8 minutes, respectively. The ZORBAX C18 column (250 x 4.6 mm, 5 µm) was chosen for the formulation of the analytical methodology. The chromatographic apparatus was continuously scrutinized utilizing Empower software. Analytes were discerned via UV absorption at 280 nm in an isocratic configuration, employing a mobile phase consisting of Acetate Buffer and Methanol in a 10:90 volumetric ratio, while sustaining a flow rate of 0.7 mL/min. The flow rate was subsequently adjusted to 1.0 mL/min, and the effluent was evaluated at 280 nm. The operational temperature and the duration of the chromatographic run were maintained at 37°C and 8 minutes, respectively.

Table3: **Optimized conditions of chromatographic work**

Parameters	Condition
stationary phase	ZORBAX C18(250*4.6,5 um)
Mobile phase	Acetate Buffer : Methanol 10:90
Flow rate	0.7 mL/min
Run Time:	8.0 Minutes
Injection volume	20.00 ul
Column Temperature	37°C
Detection wavelength	280

Preparation of standard solution

Standard stock solutions were meticulously formulated at a concentration of 200 PPM by individually solubilizing 20 mg of Sildenafil and 20 mg of Tadalafil in mobile phases, specifically a combination of acetate buffer and methanol in a proportion of 10:90, culminating in a total volume of 100 ml to produce a stock solution with an established concentration of 200 PPM. The aforementioned standard stock solution was then appropriately diluted using suitable diluents to achieve various concentrations of Sildenafil and Tadalafil, specifically 50 PPM, 80 PPM, 100 PPM, 120 PPM, and 150 PPM, for the purpose of establishing linearity.

Preparation of Sample Solutions of sildenafil and Tadalfil

A total of twenty tablets were meticulously quantified and subsequently pulverized into a fine particulate form; thereafter, the powdered tablet material corresponding to 20 mg of Sildenafil and 20 mg of Tadalafil was transferred into a clean and dry 100 ml volumetric flask. A diluent was added, and the resulting mixture was subjected to sonication to guarantee thorough dissolution, after which the volume was adjusted to the calibration mark with the diluent. The resultant sample solution underwent filtration, and a suitably diluted sample solution was prepared to attain a concentration of 200 PPM for both Sildenafil and Tadalafil, which was subsequently placed into a 100 ml volumetric flask that was clean and dry. A suitable diluent was introduced, and the resultant solution was subjected to sonication until a state of complete dissolution was achieved, subsequently followed by the calibration of the volume to the designated mark using the diluent. The aforementioned sample solution was filtered and appropriately diluted to attain a concentration of 200 PPM of Sildenafil and 200 PPM of Tadalafil.

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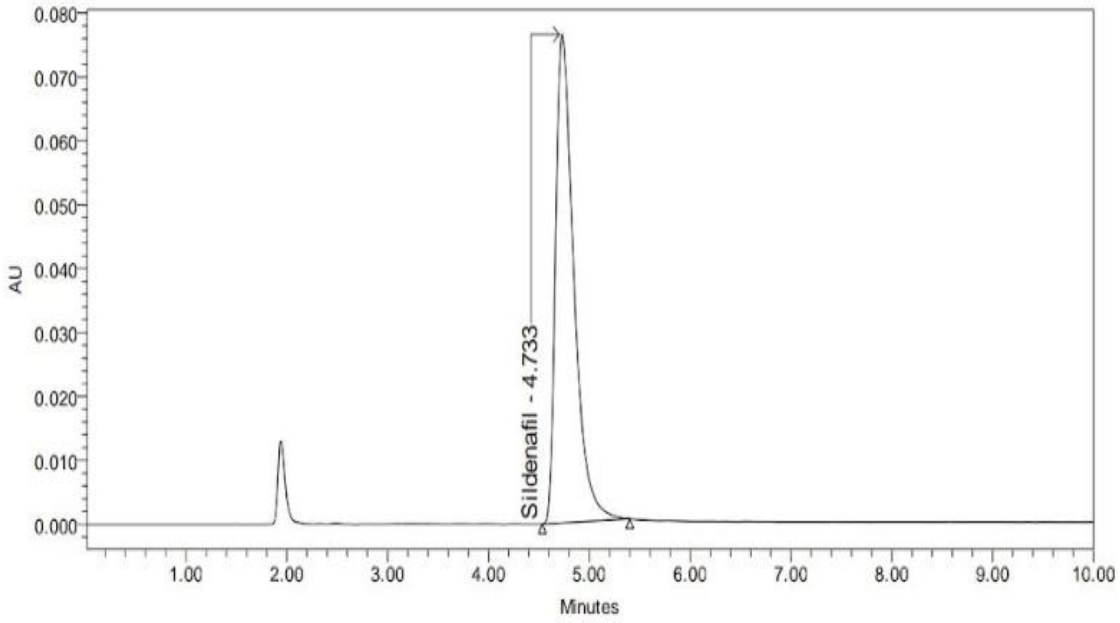
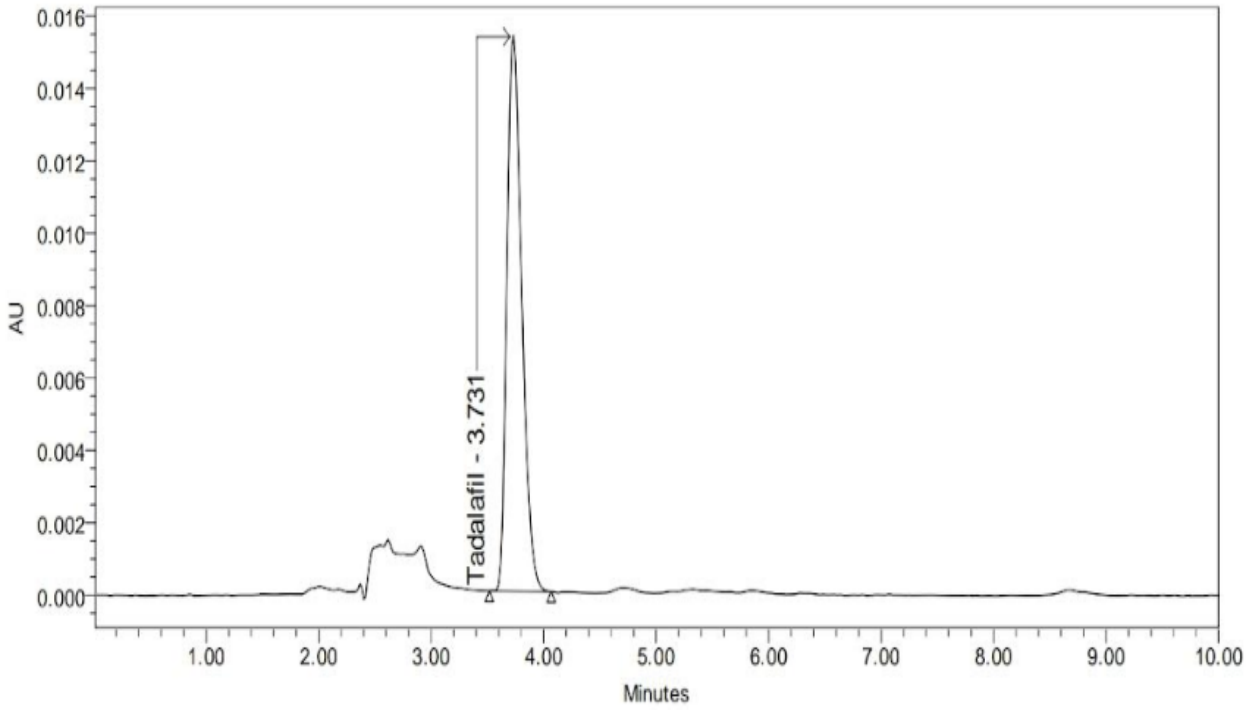


	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Tadalafil	3.747				
2	Sildenafil	4.733	963929	100.00	1.67	3356.20

Fig. 1: Normal chromatogram for Sildenafil



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

Fig. 2: Normal chromatogram for Tadalafil

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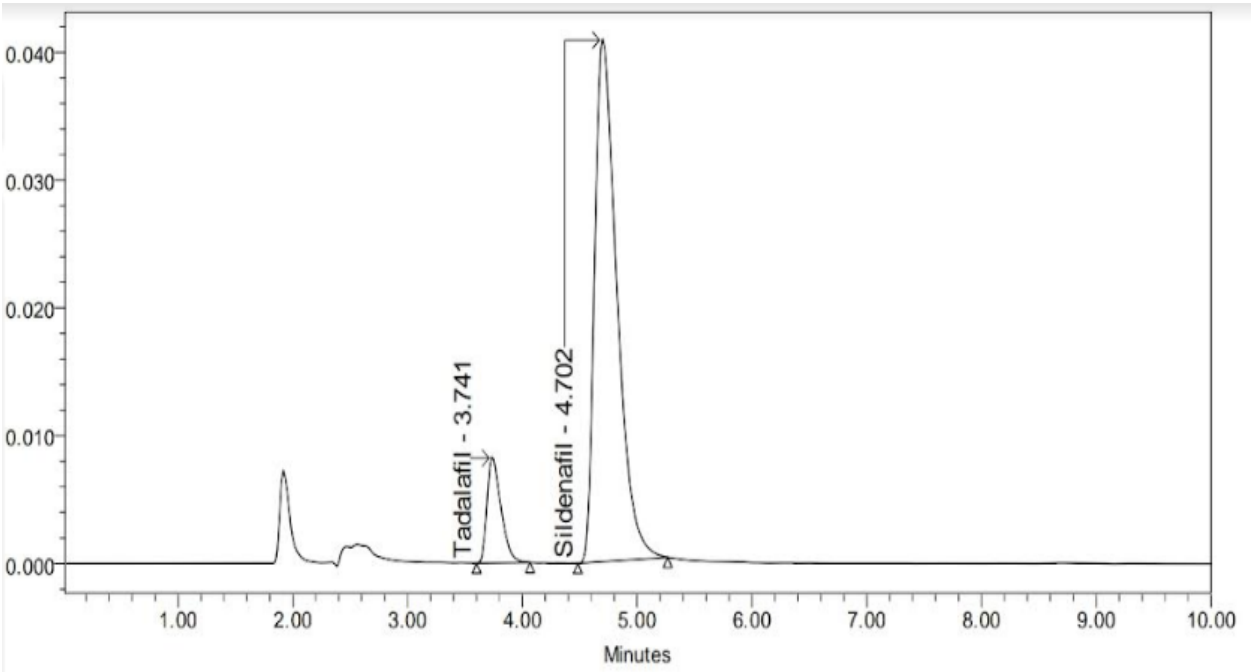
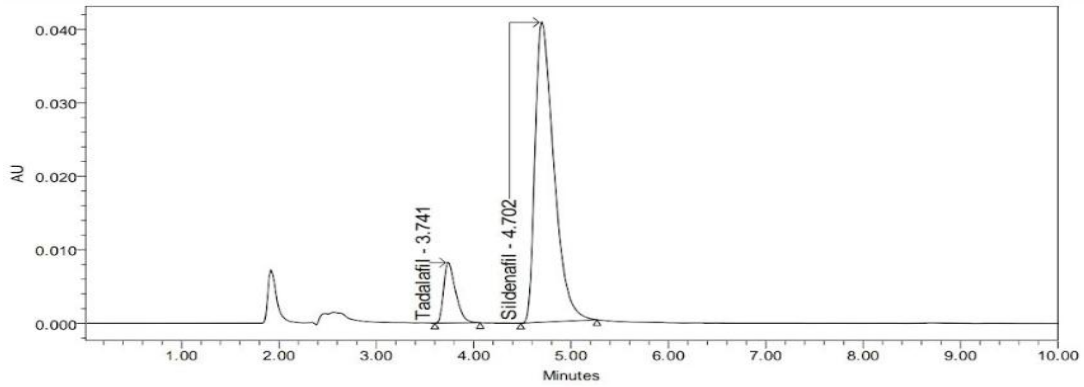


	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.741	69916	11.30	1.46	4270.63	
2	Sildenafil	4.702	549058	88.70	1.61	2783.07	3.39

Fig. 3: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

Fig. 4: Normal chromatogram for Tadalafil and Sildenafil Sample mixture

Method Validation

The methodology that was formulated has been determined to be in compliance with the validation standards established by the ICH and the FDA, with the validation parameters encompassing specificity, linearity, precision, range, accuracy, robustness, sensitivity (Limit of Quantification & Limit of Detection), and the stability of the solution.

Linearity

The ability of an analytical methodology to yield test results that are precisely commensurate with the concentration of the analyte present in the sample, within a defined range, is referred to as linearity. For single point standardization, linearity should encompass the target concentration and extend at least 20% beyond the specified range. Using peak area responses, the correlation coefficient, which defines linearity, should be 0.99.A minimum of five distinct concentrations

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of the working standard solution (50, 80, 100, 120, and 150 PPM) were prepared, and three replicates of each concentration were then made in order to achieve linearity.

Table 4: Linearity for Tadalfil

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

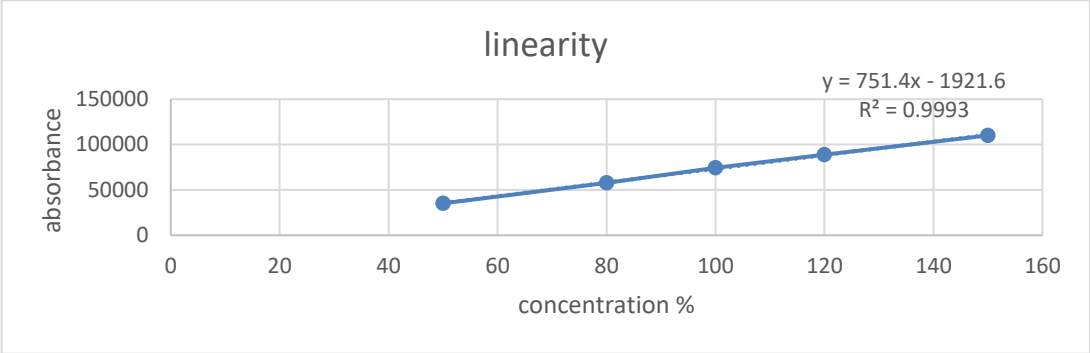

Fig 5 : Linearity for Tadalafil

Table 5: Linearity for Sildenafil

SR.NO	CONC. PPM	abs
1	50	258322
2	80	407259
3	100	517853
4	120	627931
5	150	782604

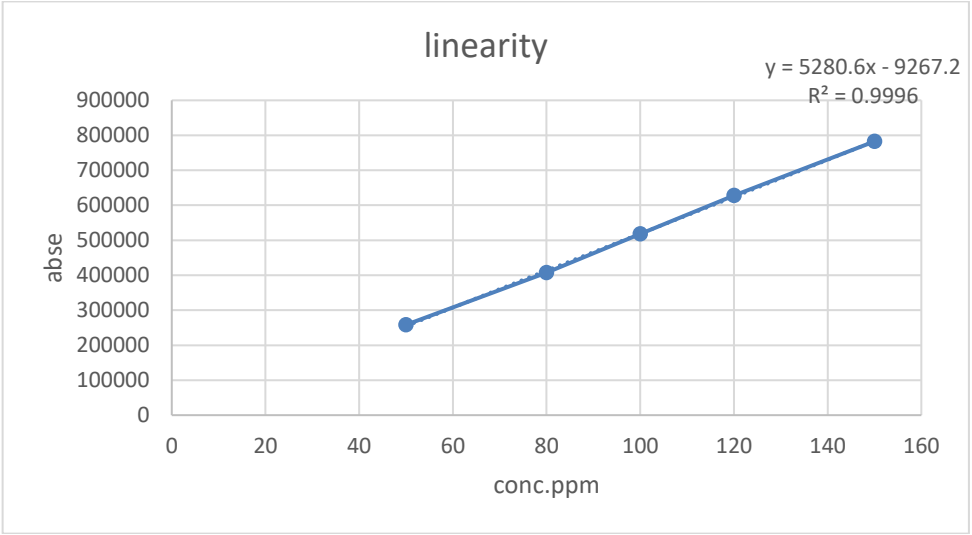

Fig 6: Linearity for Sildenafil

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Table 6 :Linearity for Sildenafil

S.NO	SampleName	Vial	Inj	Name	RetentionTime(min)	Area	%Area	USP Tailing	USP Plate count
1	Blank	15	1	Sildenafil	4.707				
2	MixStd-50ppm	16	1	Sildenafil	4.573	258322	88.01	1.71	1556.01
3	MixStd-80ppm	17	1	Sildenafil	4.566	407259	87.55	1.70	1525.97
4	MixStd-100ppm	18	1	Sildenafil	4.565	517853	87.46	1.73	1509.28
5	MixStd-120ppm	19	1	Sildenafil	4.561	627931	87.63	1.77	1527.55
6	MixStd-150ppm	20	1	Sildenafil	4.559	782604	87.67	1.78	1527.88
Mean					4.588	518793.8			
Std.Dev.					0.058	201117.7			
%RSD					1.27	38.77			

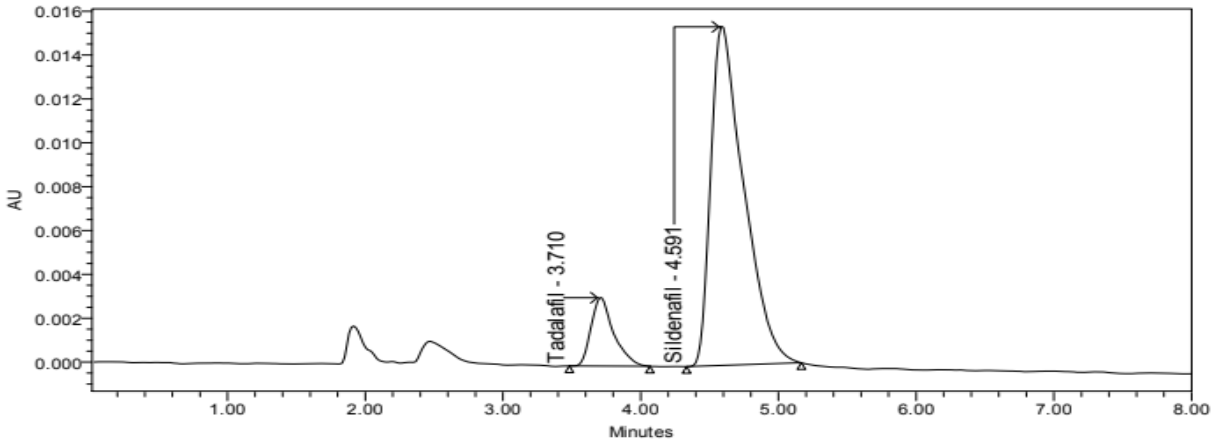
Table 7 : Linearity data for Tadalafil

SR.NO	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USP Tailing	USP Plate Count
1	Blank	15	1	Tadalafil	3.747				
2	MixStd-50ppm	16	1	Tadalafil	3.706	35176	11.99	1.36	2706.47
3	MixStd-80ppm	17	1	Tadalafil	3.705	57932	12.45	1.39	2608.58
4	MixStd-100ppm	18	1	Tadalafil	3.705	74280	12.54	1.42	2559.73
5	MixStd-120ppm	19	1	Tadalafil	3.706	88656	12.37	1.45	2414.58
6	MixStd-150ppm	20	1	Tadalafil	3.706	110049	12.33	1.45	2460.28
Mean					3.713	73218.4			
Std.Dev.					0.017	28621.8			
%RSD					0.46	39.09			

Accuracy

The standard solution was spiked in order to assess accuracy. The measurements are taken at the target concentration of tadalafil and sildenafil in Sildalist tablets, as well as at appropriate intervals around this moment.

Recovery tests at three concentration levels 50%, 100%, and 150 percent in which three samples were injected from each concentration were used to assess the assay method's accuracy. For every one of the three replicate samples, the rcentage recovery of Tadalafil and Sildenafil was determined.



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.710	35479	12.09	1.39	2517.19	
2	Sildenafil	4.591	257869	87.91	1.72	1456.14	2.45

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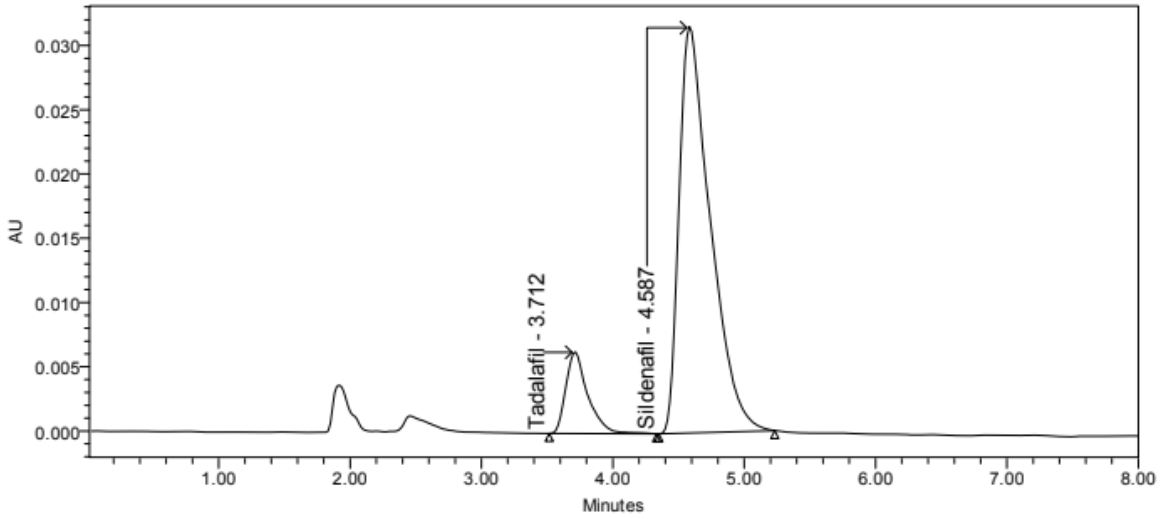
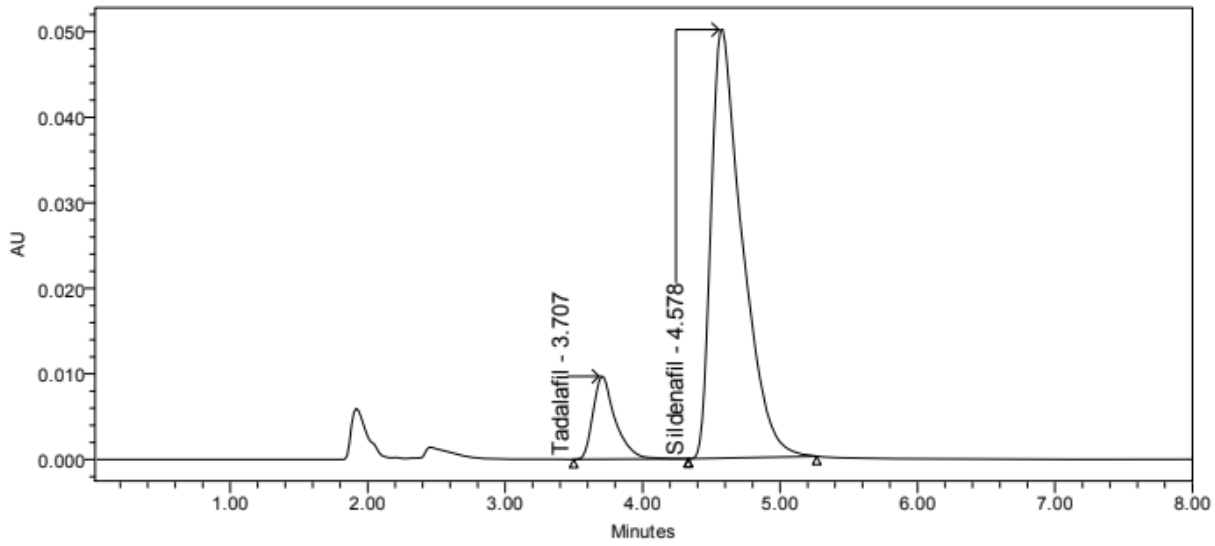
FIG 7: Chromatogram for Tadalafil and Sildenafil Accuracy 50%



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.712	73971	12.38	1.45	2439.62	
2	Sildenafil	4.587	523414	87.62	1.74	1530.54	2.45

FIG 8: Chromatogram for Tadalafil and Sildenafil Accuracy 100 %



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.707	108588	12.09	1.44	2699.59	
2	Sildenafil	4.578	789296	87.91	1.74	1765.45	2.58

FIG9:Chromatogram for Tadalafil and Sildenafil Accuracy 150%

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Table 8: Accuracy data for Sildenafil

S.NO	Sample Name	Vial	Inj	Name	RetentionTime (min)	Area	%Area	USP Tailing	USP Plate Count
1	Blank	15	1	Sildenafil	4.707				
2	Std	16	1	Sildenafil	4.571	519509	87.99	1.72	1485.70
3	Std	16	1	Sildenafil	4.575	524152	88.00	1.75	1474.42
4	Std	16	1	Sildenafil	4.576	523255	88.03	1.74	1488.63
5	Std	16	1	Sildenafil	4.578	521382	87.92	1.72	1472.84
6	B.std	16	1	Sildenafil	4.587	526001	88.12	1.73	1621.25
7	B.std	16	1	Sildenafil	4.582	532165	88.22	1.71	1741.91
8	Std	16	1	Sildenafil	4.575	522418	87.89	1.74	1480.16
9	Accuracy50%	17	2	Sildenafil	4.590	258703	87.89	1.71	1477.73
10	Accuracy50%	17	2	Sildenafil	4.591	257869	87.91	1.72	1456.14
11	Accuracy50%	17	1	Sildenafil	4.590	257587	87.74	1.70	1482.99
12	Accuracy50%	17	1	Sildenafil	4.588	257082	88.17	1.69	1471.55
13	Accuracy100%	18	1	Sildenafil	4.587	523414	87.62	1.74	1530.54
14	Accuracy100%	18	2	Sildenafil	4.588	523171	88.09	1.73	1579.91
15	Accuracy100%	18	2	Sildenafil	4.587	521500	88.05	1.73	1528.96
16	Accuracy100%	18	1	Sildenafil	4.590	522554	87.72	1.74	1552.58
17	Accuracy150%	19	1	Sildenafil	4.579	784060	87.85	1.76	1656.85
18	Accuracy150%	19	1	Sildenafil	4.574	787677	87.89	1.76	1765.48
19	Accuracy150%	19	2	Sildenafil	4.578	789296	87.91	1.74	1765.45
20	Accuracy150%	19	2	Sildenafil	4.575	787852	87.89	1.76	1701.86
Mean					4.588	523139.2			
Std.Dev.					0.029	176490.8			
%RSD					0.63	33.74			

Table 9 : Accuracy data for Tadalfil

S.NO	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USP Tailing	USP Plate count
1	Blank	15	1	Tadalafil	3.747				
2	Std	16	1	Tadalafil	3.707	70924	12.01	1.35	2495.12
3	Std	16	1	Tadalafil	3.710	71491	12.00	1.37	2451.10
4	Std	16	1	Tadalafil	3.710	71984	12.11	1.35	2443.55
5	Std	16	1	Tadalafil	3.710	71654	12.08	1.35	2430.43
6	B.std	16	1	Tadalafil	3.712	70887	11.88	1.38	2594.22
7	B.std	16	1	Tadalafil	3.705	71079	11.78	1.37	2674.47
8	Std	16	1	Tadalafil	3.710	71175	11.97	1.36	2457.00
9	Accuracy50%	17	2	Tadalafil	3.712	35656	12.11	1.38	2444.33
10	Accuracy50%	17	2	Tadalafil	3.710	35479	12.09	1.39	2517.19
11	Accuracy50%	17	1	Tadalafil	3.710	35981	12.26	1.36	2502.78
12	Accuracy50%	17	1	Tadalafil	3.711	34509	11.83	1.35	2578.10
13	Accuracy100%	18	1	Tadalafil	3.712	73971	12.38	1.45	2439.62
14	Accuracy100%	18	2	Tadalafil	3.712	70701	11.91	1.38	2578.28
15	Accuracy100%	18	2	Tadalafil	3.712	70798	11.95	1.37	2552.37
16	Accuracy100%	18	1	Tadalafil	3.713	73129	12.28	1.42	2499.99
17	Accuracy150%	19	1	Tadalafil	3.709	108398	12.15	1.46	2605.04
18	Accuracy150%	19	1	Tadalafil	3.704	108482	12.11	1.46	2703.43
19	Accuracy150%	19	2	Tadalafil	3.707	108588	12.09	1.44	2699.59
20	Accuracy150%	19	2	Tadalafil	3.706	108556	12.11	1.45	2645.74
Mean					3.711	71760.1			
Std.Dev.					0.009	24381.1			
%RSD					0.24	33.98			

High flow variation

Band broadening and decreased column efficiency can result from high flow liquid chromatography(HPLC). A portion of the sample lags behind at high flow rates because the analytic adsorbs to the stationary phase. Band broadening may

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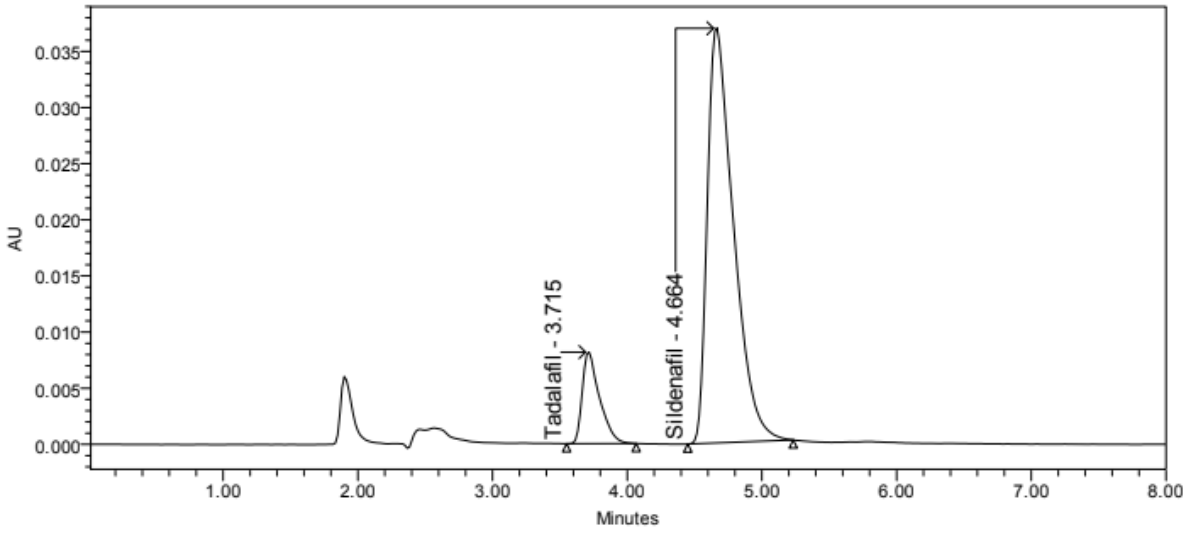
result from this. High pressure, which can be brought on by leaks or air in the pump heads, can also result from high flow rates..



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.715	72606	12.69	1.60	3807.33	
2	Sildenafil	4.664	499756	87.31	1.68	2517.22	3.30

FIG10:Chromatogram for High flow variation

Table 10 : High flow variation Data for Sildenafil

S. no.	Sample Name	Vial	Inj	Name	RetentionTime(min)	Area	%Area	USPTailing	USPPlateCount
1	bLANK	60	1	Sildenafil	4.707				
2	STD	61	1	Sildenafil	4.266	500539	88.85	1.67	2603.91
3	STD	61	4	Sildenafil	4.272	501786	88.77	1.70	2589.91
4	STD	61	2	Sildenafil	4.267	503425	88.79	1.69	2555.72
5	STD	61	3	Sildenafil	4.271	501535	88.67	1.69	2598.47
6	STD	61	5	Sildenafil	4.279	501794	88.76	1.71	2480.66
Mean					4.344	501816.1			
Std.Dev.					0.178	1036.8			
%RSD					4.10	0.21			

Table 11 :High flow variation Data for Tadalafil

S.No	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USP Tailing	USP Plate Count
1	bLANK	60	1	Tadalafil	3.747				
2	STD	61	1	Tadalafil	3.398	62783	11.15	1.51	4371.51
3	STD	61	5	Tadalafil	3.408	63531	11.24	1.62	4160.89
4	STD	61	3	Tadalafil	3.405	64091	11.33	1.61	4269.37
5	STD	61	2	Tadalafil	3.397	63527	11.21	1.58	4199.26
6	STD	61	4	Tadalafil	3.403	63458	11.23	1.59	4209.03
Mean					3.460	63478.0			
Std.Dev.					0.141	465.0			
%RSD					4.07	0.73			

LOD & LOQ

The threshold at which an analyte present within a sample can be recognized, yet not consistently quantified with a precise numerical value, is designated as the detection limit pertinent to a particular analytical procedure. The limit of

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Received: 02/08/2024 Revised:17/08/2024 Accepted: 02/09/2024 Published: 20/09/2024

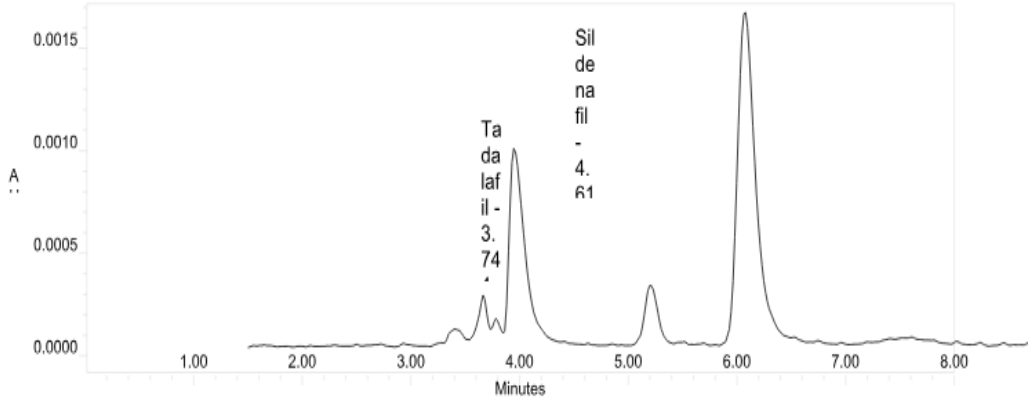
detection (LOD) can be ascertained through the equation L = 3.3 × σ/S, where σ denotes the variability or standard deviation of the calibration curve intercepts. S represents the gradient of the linear response plot. This phenomenon occurs because the assessment of the detection limit can be executed through visual inspection. The signal-to-noise ratio constitutes the comparative measure of signal strength against background noise; it encompasses both the slope and dispersion of the response function.

The quantitation threshold for any particular analytical technique is defined as the lowest concentration of the analyte that can be consistently identified within a sample and quantified with an adequate level of accuracy and precision. In instances involving trace quantities of chemical substances within a sample matrix, the limit of quantitation serves as a critical parameter in quantitative assessments, particularly utilized for the identification and quantification of pollutants and their degradation products. The limit of quantitation, under specified conditions, may be determined through the application of the following equation:

Where, δ = standard deviation of response LOQ = 10 × δ/S

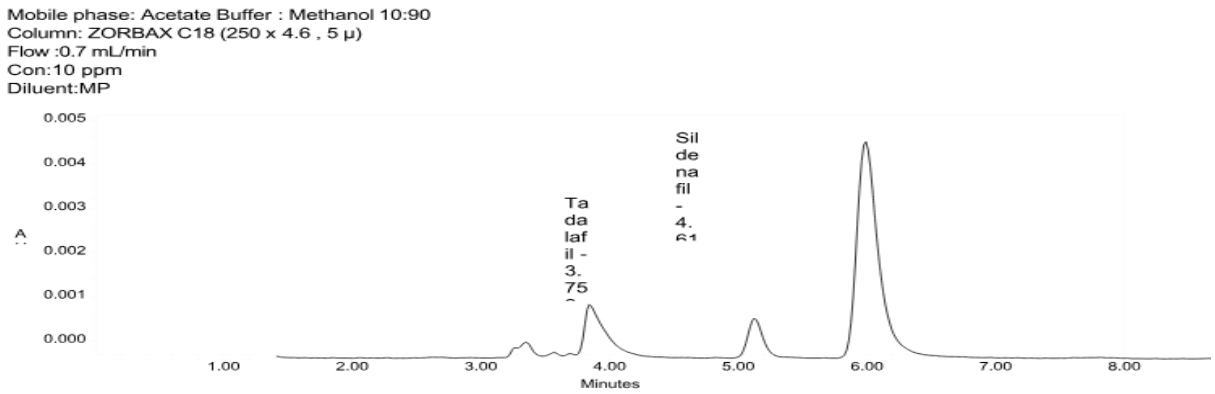
S = Average of slope of the calibration curves.

Through the application of progressively reduced volumes of the standard solutions within the methodologies delineated, the limits of detection (LOD) and limits of quantification (LOQ) for the novel analytical technique were ascertained. The LOQ signifies the minimum concentration of the analyte at which quantification can be performed with a degree of accuracy and precision that is deemed satisfactory, while considering the signal-to-noise ratio; conversely, the LOD represents the minimum concentration that can be identified and differentiated, also taking into account the signal-to-noise ratio. The established LOD values for both Tadalafil and sildenafil were determined to be 1.65 parts per million (PPM). Furthermore, Tadalafil and sildenafil were evaluated to possess a LOQ of 5 PPM.



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	s/n
1	Tadalafil	3.744	2385	12.43	1.24	4872.75	84
2	Sildenafil	4.611	16812	87.57	1.22	4142.76	466

Fig 11: Normal chromatogram LOD & LOQ for std



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	s/n
1	Tadalafil	3.752	7055	11.51	1.16	4997.10	157
2	Sildenafil	4.614	54257	88.49	1.37	3959.96	854

Fig 12:Normal chromatogram LOD & LOQ for sample

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Received: 02/08/2024 Revised:17/08/2024 Accepted: 02/09/2024 Published: 20/09/2024

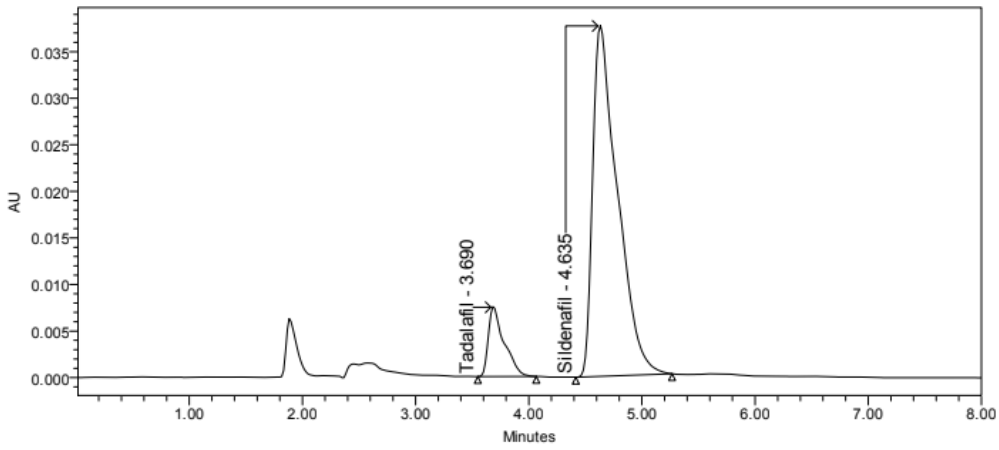
Table 12: LOD & LOQ Of Sildenafil and Tadalfil

	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USP Tailing	USPPlate Count
1	Blank	60	1	Sildenafil	4.707				
2	Std	61	1	Sildenafil	4.607	530169	88.79	1.60	4575.38
3	Std	61	1	Sildenafil	4.607	529852	88.78	1.60	4544.34
4	Std	61	1	Sildenafil	4.600	526627	88.76	1.59	4503.01
5	Std	61	1	Sildenafil	4.601	528059	88.70	1.59	4532.10
6	Std	61	1	Sildenafil	4.605	526954	88.78	1.60	4527.37
7	LOQPrecision	62	5	Sildenafil	4.599	54520	88.64	1.35	4025.85
8	LOQPrecision	62	4	Sildenafil	4.598	54323	88.82	1.35	4026.30
9	LOQPrecision	62	2	Sildenafil	4.603	55097	88.87	1.38	4003.02
10	LOQPrecision	62	1	Sildenafil	4.605	55051	88.83	1.39	3992.06
11	LOQPrecision	62	3	Sildenafil	4.602	54487	88.66	1.34	3947.65
12	LOQ5ppm	62	1	Sildenafil	4.614	54257	88.49	1.37	3959.96
13	LOQ7ppm	63	1	Sildenafil	4.609	74820	88.53	1.39	4045.58
14	LOQ10ppm	64	1	Sildenafil	4.604	106863	88.77	1.43	4084.65
15	LOD1.65ppm	65	1	Sildenafil	4.611	16812	87.57	1.22	4142.76
Mean					4.611	226277.9			
Std.Dev.					0.027	234367.2			
%RSD					0.58	103.57			
	SampleName	Vial	Inj	Name	RetentionTime(min)	Area	%Area	USP Tailing	USP Plate Count
1	Blank	60	1	Tadalafil	3.747				
2	Std	61	1	Tadalafil	3.772	66906	11.21	1.27	7373.52
3	Std	61	1	Tadalafil	3.772	66627	11.22	1.28	7375.34
4	Std	61	1	Tadalafil	3.770	67255	11.30	1.27	7460.49
5	Std	61	1	Tadalafil	3.770	66680	11.24	1.27	7533.31
6	Std	61	1	Tadalafil	3.774	66972	11.22	1.27	7336.34
7	LOQPrecision	62	5	Tadalafil	3.731	6989	11.36	1.15	4800.14
8	LOQ5ppm	62	1	Tadalafil	3.752	7055	11.51	1.16	4997.10
9	LOQPrecision	62	4	Tadalafil	3.731	6837	11.18	1.14	4942.37
10	LOQPrecision	62	3	Tadalafil	3.734	6967	11.34	1.16	4731.83
11	LOQPrecision	62	1	Tadalafil	3.738	6925	11.17	1.17	4856.98
12	LOQPrecision	62	2	Tadalafil	3.736	6898	11.13	1.16	4994.09
13	LOQ7ppm	63	1	Tadalafil	3.748	9694	11.47	1.16	5122.60
14	LOQ10ppm	64	1	Tadalafil	3.746	13525	11.23	1.17	5376.03
15	LOD1.65ppm	65	1	Tadalafil	3.744	2385	12.43	1.24	4872.75
Mean					3.751	28693.9			
Std.Dev.					0.016	29633.1			
%RSD					0.44	103.27			

Intermediate Precision

Intermediate precision pertains to the performance of the methodology, encompassing both qualitative and quantitative aspects, within a singular laboratory, while also accounting for variations between instruments and across different days, and subsequently computing the percentage relative standard deviation (% RSD) of the assay.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Received: 02/08/2024 Revised:17/08/2024 Accepted: 02/09/2024 Published: 20/09/2024



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count	Resolution
1	Tadalafil	3.690	72453	11.22	1.79	3550.15	
2	Sildenafil	4.635	573041	88.78	1.91	1550.56	2.99

Fig 13:Normal chromatogram Intermediate Precision

Table 13:Intermediate Precision Data for Sildenafil

SR.NO	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USP Tailing	USP Plate Count
1	Blank	60	1	Sildenafil	4.707				
2	std	61	1	Sildenafil	4.631	570525	88.66	1.90	1505.37
3	std	61	1	Sildenafil	4.635	573041	88.78	1.91	1550.56
4	std	61	1	Sildenafil	4.635	572444	88.77	1.90	1636.58
5	std	61	1	Sildenafil	4.637	572930	88.76	1.93	1839.75
6	B.std	61	1	Sildenafil	4.622	578782	88.79	1.97	2675.49
7	std	61	1	Sildenafil	4.634	570888	88.78	1.94	1817.19
8	M.P.Test1	62	1	Sildenafil	4.634	561378	88.80	1.93	1895.65
9	M.P.Test2	63	1	Sildenafil	4.631	569697	88.83	1.93	1904.55
10	M.P.Test3	64	1	Sildenafil	4.631	562553	88.83	1.93	2113.00
11	M.P.Test4	65	1	Sildenafil	4.628	559283	88.80	1.95	2283.22
12	M.P.Test5	66	1	Sildenafil	4.624	578762	89.17	1.91	2766.15
13	M.P.Test6	67	1	Sildenafil	4.620	565896	88.47	1.83	4056.30
Mean					4.636	569681.7			
Std.Dev.					0.022	6318.3			
%RSD					0.47	1.11			

Method Precision - Sildenafil

	System Suitability						
	Area	R.T.		B.std			% Aassay
	570525	4.631		570525		Test-1	98.1
	573041	4.635		573041		Test-2	99.6
	572444	4.635		572444		Test-3	98.4
	570888	4.634		570888		Test-4	97.8
	572930	4.637		572930		Test-5	102.2
Avg	571966	4.635	B.std	578782		Test-6	98.9
sd	1178.1	0.0				Avg	99.2
rsd	0.21	0.03	Avg	573102		sd	1.6158
			sd	2975.6		rsd	1.63
			rsd	0.52			

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Received: 02/08/2024 Revised:17/08/2024 Accepted: 02/09/2024 Published: 20/09/2024

Table 13:Intermediate Precision Data for Sildenafil

	Samle Name	Vial	Inj	Name	Retention Time (min)	Area	%Area	USP Tailing	USP Plate count
1	blank	60	1	Tadalafil	3.747				
2	std	61	1	Tadalafil	3.688	7298	11.34	1.78	3765.97
3	std	61	1	Tadalafil	3.690	7245	11.22	1.79	3550.15
4	std	61	1	Tadalafil	3.690	7245	11.23	1.78	3737.86
5	std	61	1	Tadalafil	3.688	72160	11.22	1.82	4240.34
6	B.std	61	1	Tadalafil	3.686	73095	11.21	1.84	4826.72
7	std	61	1	Tadalafil	3.692	72536	11.24	1.82	3807.66
8	M.P.Test1	62	1	Tadalafil	3.690	70832	11.20	1.81	4048.09
9	M.P.Test2	63	1	Tadalafil	3.690	71643	11.17	1.81	4066.10
10	M.P.Test3	64	1	Tadalafil	3.690	70733	11.17	1.82	4345.63
11	M.P.Test4	65	1	Tadalafil	3.690	70556	11.20	1.83	4584.14
12	M.P.Test5	66	1	Tadalafil	3.688	70267	10.83	1.66	5180.63
13	M.P.Test6	67	1	Tadalafil	3.689	73785	11.53	1.77	6245.09
Mean					3.694	71958.2			
Std.Dev.					0.016	1137.8			
%RSD					0.44	1.58			

Table 14: Intermediate Precision Data for Tadalfil

Intermediate Precision - Tadalafil

	System Suitability						
	Area	R.T.		B.std			% Aassay
	72984	3.688		72984		Test-1	97.7
	72453	3.69		72453		Test-2	98.8
	72160	3.688		72160		Test-3	97.5
	72536	3.692		72536		Test-4	97.3
	72453	3.69		72453		Test-5	96.9
Avg	72517	4	B.std	73095		Test-6	96.9
sd	297.5	0.0				Avg	97.5
rsd	0.41	0.04	Avg	72614		sd	0.7055
			sd	355.6		rsd	0.72
			rsd	0.49			

Solution stability

The concept of solution stability pertains to the degree of stability exhibited by both the standard solution and the extracted sample solution, or the solution prepared for injection derived from the sample or matrix, and analyzed in accordance with the established methodology. The standard and sample solutions ought to be maintained under room temperature conditions or refrigerated environments, contingent upon the inherent stability characteristics of the standard and sample solutions.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
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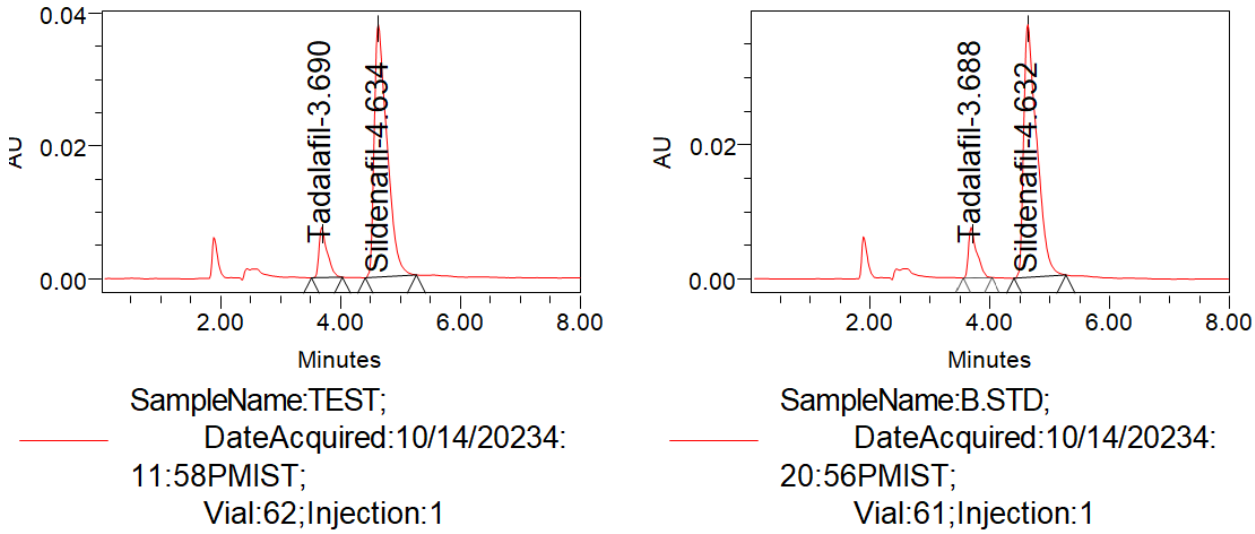

Fig 14:Normal chromatogram Solution stability

Table 15 : Solution stability Sildenafil

SR.NO	Sample Name	Vial	Inj	Name	Retention Time(min)	Area	%Area	USPTailing	USPPlateCount
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
Mean					4.639	571290.5			
Std.Dev.					0.028	4410.9			
%RSD					0.59	0.77			

Table 16 : Solution stability Tadalafil

SR.NO	Sample Name	Vial	Inj	Name	RetentionTime(min)	Area	%Area	USPTailing	USPPlateCount
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
Mean					3.695	71842.4			
Std.Dev.					0.021	604.7			
%RSD					0.57	0.84			

Wavelength variation

More importantly, in order to optimize the fluorescent funnel, the absorption spectrum of the molecule of interest must be examined. This reduces the spectral interference produced by the error in measurement of the detector alone.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
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Table 17: Wavelength variation

Wavelength Variation - Sildenafil - 278 nm			Wavelength Variation - Sildenafil -282 nm		
	System Suitability			System Suitability	
	Area	R.T.		Area	R.T.
	633099	4.674		499756	4.664
	631880	4.684		498540	4.668
	632273	4.685		498682	4.672
	630270	4.685		499589	4.666
	632241	4.682		499019	4.667
Avg	631953	4.684	Avg	499117	4.668
sd	1041.2	0.0	sd	539.2	0.0
rsd	0.16	0.03	rsd	0.11	0.06

Wavelength Variation - Sildenafil - 278 nm			Wavelength Variation - Sildenafil -282 nm		
	System Suitability			System Suitability	
	Area	R.T.		Area	R.T.
	633099	4.674		499756	4.664
	631880	4.684		498540	4.668
	632273	4.685		498682	4.672
	630270	4.685		499589	4.666
	632241	4.682		499019	4.667
Avg	631953	4.684	Avg	499117	4.668
sd	1041.2	0.0	sd	539.2	0.0
rsd	0.16	0.03	rsd	0.11	0.06

Dissolution study

The novel high-performance liquid chromatography (HPLC) methodology employed for the examination of dissolution samples pertaining to the pharmaceutical formulations demonstrated its proficiency in effectively segregating the primary peak from the confounding peaks. Loss due to degradation was handled by using dissolution media to perform dissolution analysis.

Table 19: Dissolution study

	Std	Disso spl
	0.759	0.793
	0.758	0.789
	0.758	
Average	0.758	0.791
SD	0.000577	
RSD	0.076134	

Results of assay of marketed formulation

By conducting a mass determination of twenty tablets and calculating their mean weight, the mass of an individual tablet was subsequently transferred into a 100mL volumetric flask, followed by the addition of 50mL of diluents, after which the flask was subjected to sonication for a duration of 25 minutes and subsequently filtered. A volume of one millilitre from the filtered solution was then carefully pipetted into a 10 millilitre volumetric flask, and the remaining volume was adjusted to the mark with the same diluents.

Table 20: Assay of marketed formulation

Brand	Drug	Sample peak area	Standard peak area	Labelled amount (mg/tab)	% Assay	RSD
Sildalist	Sildenafil	789296	799456	100	99.73	0.91
	Tadalfil	108588	108690	20	100.45	1.21

Conclusion

An uncomplicated, exacting, and exceptionally sensitive RP-HPLC technique is articulated for the simultaneous quantification of Sildenafil and Tadalafil in pharmaceutical formulations. The method yields significant resolution

between the analytes within a concise analytical duration. Ultimately, it was concluded that the technique is suitable for the evaluation of standard quality control samples.

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