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A Stability Indicating RP-UPLC method for Simultaneous Determination of Sildenafil and Tadalafil in Bulk Drugs and Pharmaceutical Dosage Forms.

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ABSTRACT

This study is aimed at Developing and validating an UPLC method for determination of Sildenafil and tadalafil content in API and formulations. A chromatographic system consisting Waters Acquity UPLC BEH C8(1.8 µm)column, mobile phase of 0.2 M ammonium acetate and Acetonitrile with gradient elution at flow of 0.3 mL/min and UV detector set at 245 nm has shown a good chromatographic separation for Sildenafil tadalafil. The developed method was validated as per ICH Guidelines, the developed UPLC method has run time of only 10 minutes making the method productive and tested by spiking all the impurities of sildenafil and tadalafil. It may be applied for Quality control Testing.

Keywords: Sildenafil citrate, Tadalafil, Stability indicating, RP-UPLC.

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INTRODUCTION

Sildenafil citrate[1] is a therapy used for erectile dysfunction by acting as a selective inhibitor of cyclic guanosine mono phosphate(cGMP)-specific phosphor diesterase type 5(PDES).Sildenafil is chemically known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 H pyrazolo[4,3-d]pyrimidine-5-yl)-4-ethoxy phenyl]sulfonyl]-4-methyl piperizine citrate has the following structural formulae$C_{28}H_{38}N_6O_{11}S$. And its molecular mass: base: 474.6 g mol−1; citrate: 666.7 g mol−1. Sildenafil enhances relaxation of the corpus cavernosal smooth muscle, which in turn increases blood flow into the cavernosal spaces, thus leading to increased intra cavernosal pressure, a key factor in producing an erect penis[2-3].

Sildenafil citrate, sold under the names Viagra, was a drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH). However, the introduction of sildenafil resulted to its widespread use as well as its abuse. Therefore, specific, accurate, and robust determination of this drug is widely required. Several methods have been developed for this purpose. Pistoset at al [4] have proposed a HPLC method for determination of sildenafil and its active metabolite (N-desmethyl sildenafil) in human blood. Determination of sildenafil citrate in human plasma [5-9] and in pharmaceutical formulations[10-15] using chromatographic methods has been reported.

Tadalafil (Cialis®) is used in oral treatment for erectile dysfunction. Tadalafil is chemically (6R, 12aR)-2, 3, 6, 7, 12, 12a-hexahydro-2-methyl-6-(3,4-methylene dioxyphenyl) pyrazino (1′, 2′: 1,6) pyrido- (3,4-b) indole-1, 4-dione. Tadalafil is a white crystalline solid that melts at approximately 301-302°C. It is practically insoluble in water and very slightly soluble in ethanol. The empirical formula of tadalafil is $C_{22}H_{19}N_3O_4$. The molecular weight of Tadalafil is 389.4.

Several analytical methods that have been reported for the estimation of TDF in biological fluids or pharmaceutical dosage forms include liquid chromatography [16-19], densitometry[20] and spectrophotometry [21-22.]

Sildalis - a combination of tadalafil and sildenafil citrate goes to work for typical sexual problems. Sildalis power packed with Tadalafil and Sildenafil citrate can handle the most severe problem of erectile dysfunction so that you can be the extra stronger for your mistress. Sildalis slither up to her without a worry and perform with complete confidence with tadalafil and Sildenafil citrate of fire power. The sildalis tablet is administered orally to treat erectile dysfunction and impotency in men. Red film coated rectangular shaped tablets packed in a pocket size blister strip of 6 tablets comes in tadalafil 20mg and Sildenafil citrate 100mg. Most

men who used sildalis had the ability to do better for an extended amount of time without concerns about losing their erections. This tablet provides a prolonged effect compared to the standard red tablets in market. As a result sexual arousal in harsh, prolonged erection for maximum sexual performance.

The literature survey reveals that there are some methods available for estimation of both sildenafil and tadalafil with single method of analysis [23]; the method was longer runtime and lack of specificity by impurities. Ultra performance liquid chromatography (UPLC) is a new category of separation science which builds upon well established principles of liquid chromatography, using sub 2 µm porous particles. These particles operate at elevated mobile phase velocities to produce rapid separations with increased sensitivity and increased resolution. Thus UPLC technology allows analysts time to be drastically reduced while still meeting assay acceptance criteria based on plate count, resolution and analyte retention.

The HPLC method of analysis as reported in the pharmacopoeia is adequate to separate all the process related as well as the probable degradation related substances in both the API as well as finished dosage form. However, this method suffers from an inordinately long run time. The current paper describes the development and validation of method for estimation of sildenafil and tadalafil by UPLC method which separates all the impurities within a short span of 10 minutes run time. The method equivalency with the Pharmacopeia method was established for both the compounds.

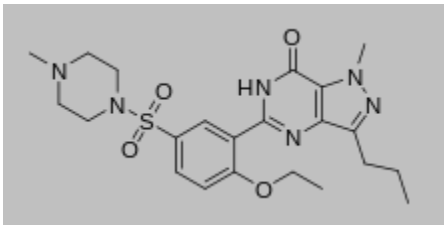

Figure1: Sildenafil citrate chemical structure

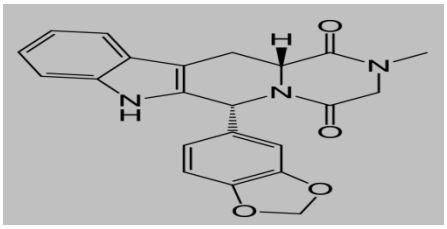

Figure 2: Tadalafil chemical structure.

MATERIALS AND METHODS:

Instruments:

A Waters acquity ultra performance liquid chromatography equipped with PDA Detector with Binary pump. The column utilized was Acquity UPLC, BEH, C8, 2.1x100mm, 1.8µm.

Chemicals:

All the chemicals used were of pharmaceutical grade. Acetonitrile of chromatographic grade and

extra pure ammonium acetate were from Merck. Sildenafil citrate, its impurities obtained from MSN Labs Hyderabad, Tadalafil, Its impurities were obtained from SMS Labs, Hyderabad, Viagra tablets were obtained from Pfizer, Cialis tablets are obtained from Eli Lilly.

1) Sildenafil amino impurity: 4-amino-1-methyl-3-propyl-1H-pyrazole-5-carboxamide.

2) Sildenafil adduct impurity: 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6, 7, dihydro-1H-pyrazolo [4, 3-d] pyrimidin-5-yl) benzene-1-sulfonyl chloride.

3) Sildenafil coupled impurity: 4-(2-ethoxy benzamido-1-methyl-3-propyl-1H-pyrazole-5-carboxamide..

4) Sildenafil cyclised impurity: 5-(2-ethoxy phenyl)-1-methyl-3-propyl-1H-pyrazino [4, 3,-d] pyramidin-7(6H)-one.

5Tadalafil impurity-A: Benzo[d] [1,3]dioxole-5-carbaldehyde.

6) Tadalafil impurity-B:{6R-cis)-6-(1,3-benzodioxo(-5-yl)-2,3,6,7,12,12a-hexa hydro-2-methyl pyrazino[1,2:1,6]pyrido[3,4-b]-indole-1,4-dione.

7)Tadalafil impurity-C(1R,3R)-methyl-1-(benzo[d][1,3]dioxol-5-yl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro1H-pyrido[3,4-b]indole-3-carboxylate.

Developing an UPLC Method:

The UPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Sildenafil and tadalafil from its process related impurities and degradation products that comply with the general requirements for system suitability. Initial trials were done with 1mL of tri flouro acetic acid in 1L, and acetonitrile gradient at flow rate 0.5 mL·min–1. Poor peak shape of Sildenafil and resolution with tadalafil impurity-2 and impurity-3 was the problem.

Different columns such as BEH C_{18}, BEH C_8 and different buffers such as formic acid, trifluoroacetic acid, KH_2PO_4 were also tried with different isocratic and gradient methods to achieve the best chromatographic separation. But long retention times and poor peak shapes were still unavoidable.

With 0.1% tri fluoro acetic acid, Tadalafil impurities are co-eluting and long retention times are seen. Studied the separation and peak shape by varying pH from 2.5 to 7.0 with phosphate buffer, and observed that, as the pH is decreasing towards 3.0, peaks were strongly retaining. Also at higher pH, Sildenafil coupled impurity and Tadalafil are co eluting. Added ammonium acetate to the buffer phase and acetonitrile to study the separation on a BEH C_8, and 100mm column at 6.6 pH. The resolution and peal shapes were significantly improved. After many logical gradient adjustments, chromatographic condition was established such that which could

be suitable for separation of drug degradation products and seven known impurities. Using the optimized conditions, Sildenafil, tadalafil and its known impurities were well separated with a resolution of greater than 1.5.

Finalized conditions:

The chromatographic column used was Acquity, UPLC, BEH, C-8, and Column (100 × 2.1) mm with 1.8 µm particles. Buffer consists of a mixture of 0.2 M Ammonium acetate as aqueous phase and Acetonitrile as organic phase with the gradient programme (Table-1). The flow rate of the mobile phase was 0.4 mL·min–1. The column temperature was maintained at 40°C and the detection was monitored at a wavelength of 245 nm. The injection volume was 1µL. Buffer and acetonitrile in 1:8 ratios was used as diluent. The conc. is 100 ppm was used for Assay method.

Table 1: Gradient programme

Time(min)	Flow(mL/min)	%A	%B
0	0.4	80	20
1.5	0.4	70	30
3.5	0.4	65	35
5.5	0.4	50	50
7.5	0.4	20	80
8	0.4	80	20
10	0.4	80	20

PREPARATION OF SOLUTIONS:

Preparation of standard solution

A stock solution of Sildenafil and tadalafil (0.1 mg·mL–1) was prepared by dissolving appropriate amount in the diluent.

Preparation of Test solutions (Sildenafil):

Sildenafil citrate tablets contain 100 mg of sildenafil .Twenty tablets (100 mg) were weighed and the average weight was calculated. The tablets were powdered in a mortar and a sample of the powder equivalent to 300 mg of the active pharmaceutical ingredient (Sildenafil) was transferred to 100 mL volumetric flask. Approximately 80 mL diluent was added and the flask was placed on rotatory shaker for 10 min and sonicated for 30 min to dissolve the material completely. The solution was then diluted to 100 mL and centrifuged at 3000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm pore size Syringe filter, the above filtered solution on dilution of 3.3 mL to 100 mL (100 ppm) used as test solution for assay.

Preparation of Test solutions (Tadalafil):

Tadalafil tablets contain 20 mg of sildenafil .Twenty tablets (20 mg) were weighed and the average weight was calculated. The tablets were powdered in a mortar and a sample of the

powder equivalent to 60 mg of the active pharmaceutical ingredient (Tadalafil) was transferred to 200 mL volumetric flask. Approximately 150 mL diluent was added and the flask was placed on rotatory shaker for 10 min and sonicated for 30 min to dissolve the material completely. The solution was then diluted to 200 mL and centrifuged at 3000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm pore size Syringe filter, the above filtered solution on dilution of 3.3 mL to 10 mL (100 ppm) used as test solution for assay.

Quantification:

Equal volumes, (1µL), of the standard preparations and the test preparations that contain sildenafil and tadalafil were injected into the chromatograph and the chromatograms were recorded. The responses (peak area) for the major peaks were measured and the quantity of sildenafil and tadalafil was calculated from the equation Cs (Au / As) where Au and As are the areas under the corresponding peaks and Cs is the concentration of sildenafil and tadalafil in the standard solution.

METHOD VALIDATION

The method validation was performed as per ICH Guidelines.

Linearity:

The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope.

Precision

The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Sildenafil and tadalafil contents.

Accuracy

The samples were prepared by spiking Sildenafil and tadalafil stock solutions into the Placebo mixture and the percent recovery was estimated.

Solution stability

The solutions prepared was tested at initial, 24hrs and 48Hrs by maintaining at room temperature and estimated for Sildenafil and tadalafil content.

Robustness

Robustness was conducted by making the variations in flow rate, Column oven temperature.

Ruggedness

The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PVDF syringe filter and evaluated against the centrifuged sample.

Intermediate precision

The test was performed with another analyst on different day, different system and different column and the impurity contents were reported.

Forced degradation studies

The forced degradation studies conditions and % degradation s mentioned in the results (Table: 9) section.

Study for Uneluted peaks:

Since the runtimes are lower, a study conducted on all the stressed samples for knowing the retained peaks by increasing the acetonitrile to 90% till 15 minutes.

Equivalency with the Pharmacopeia methods:

The developed UPLC method was tested for equivalency with Pharmacopeia methods in three steps.

System suitability equivalence:

The System suitability parameters in the pharmacopeia methods and develop method are compared with the obtained values.

API Analysis equivalence:

The results obtained with the Same API batch analysis with the pharmacopeia methods and the developed method, results were discussed.

Reference Product analysis equivalence:

The results obtained with the Same Viagra and cialis tablet batch analysis with the pharmacopeia methods and the developed method, results were discussed.

RESULTS AND DISCUSSIONS

The impurity mix, Blank, Placebo chromatograms was obtained after finalization of method was as below

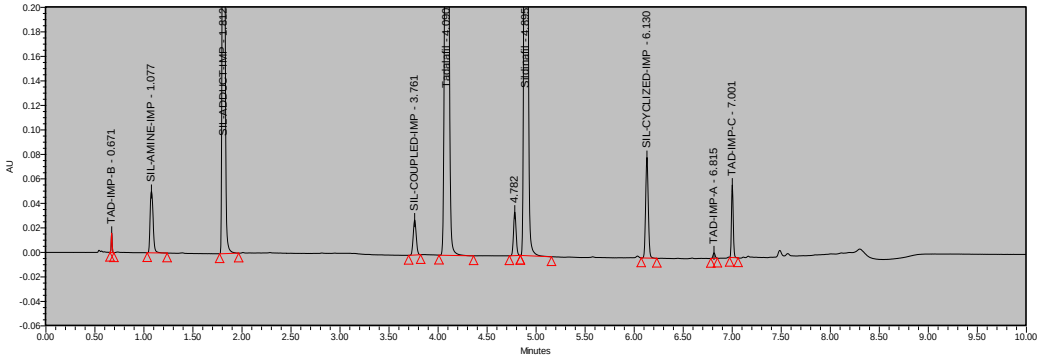

Figure 3: Impurities spiked sample chromatogram.

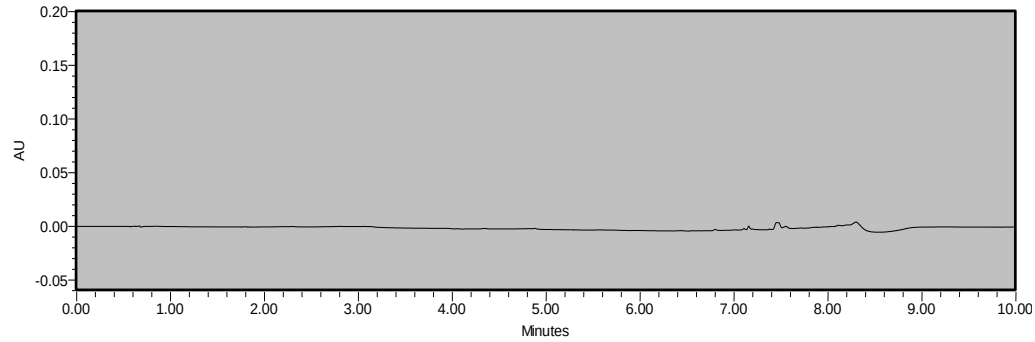

Figure 4: Blank chromatogram.

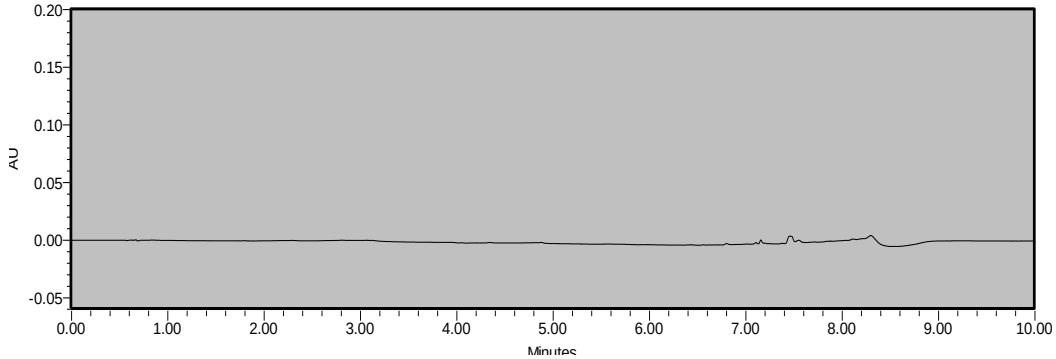

Figure 5: Placebo chromatogram.

Assay method validation results:

Specificity:

Blank interference:

The diluent was injected as a blank; it was found that there was no interference observed in placebo preparation with the Sildenafil and tadalafil peaks.

Placebo interference:

With the equivalent weight of sample the blank preparation was prepared and injected into the system and interference checked, it was found that here was no interference observed in placebo preparation with the Sildenafil and tadalafil peaks.

Impurity interference:

Impurity solution was prepared at 3 % level of test concentration and injected into the system and checked for the interference, it was found that impurities are not interfering with the Sildenafil and tadalafil peaks..

Linearity:

The linearity was performed at 6 levels of the targeted 100 ppm (10%, 25%, 50%, 100%, 200%, 300% levels) and the area results are plotted against the concentration, the correlation coefficient observed was 1 for sildenafil and tadalafil.

Table 2: Sildenafil linearity values

% level	Conc.	Avg.area
10	10.01	44357
25	25.03	109249
50	50.06	219274
100	100.12	437618
200	200.24	883018
300	300.36	1333962

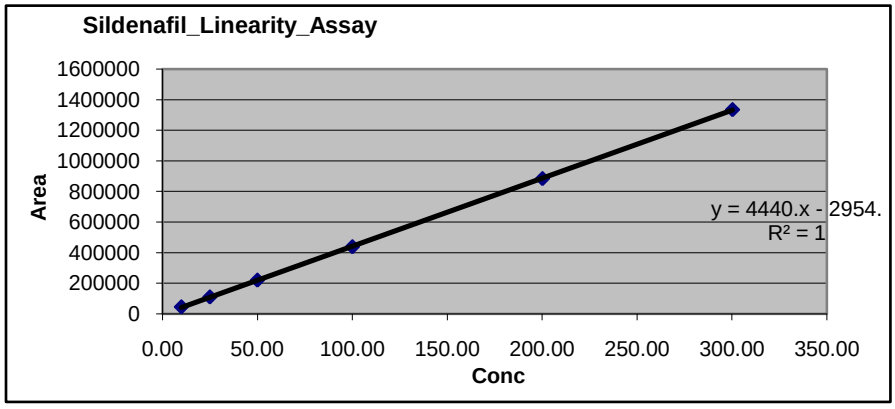

Figure6: Sildenafil linearity plot

Table3: Tadalafil linearity values

% level	Conc.	Avg.area
10	10.02	37182
25	25.06	91866
50	50.12	183456
100	100.24	366427
200	200.48	727787
300	300.72	1097589

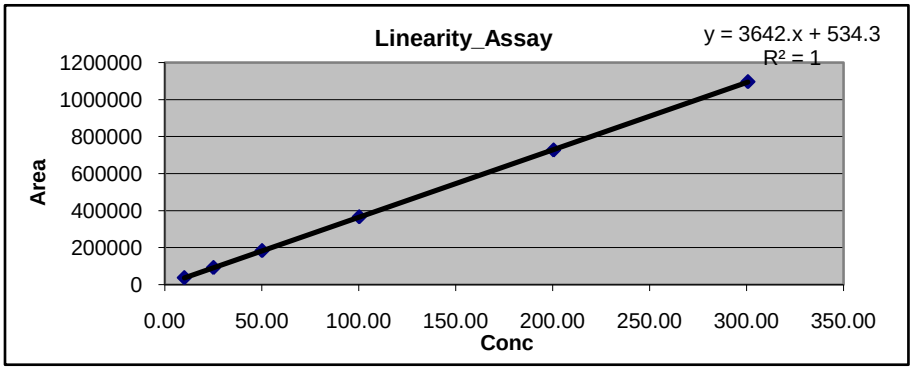

Figure 7: Tadalafil linearity plot

Precision:

By following the procedure in 2.5.2 &2.5.3 sections, six sample preparations are prepared and calculated the assay values and the Percent relative standard deviation was 0.19 for sildenafil and 0.28 for tadalafil shows that the method was precise as per the ICH limits.

Table 4: Tadalafil precision Results

S.No	Area-1	Area-2	Mean	% Assay	Mean	SD	%RSD
1	368320	367988	368154.0	101.62	101.24	0.28	0.28
2	366316	367142	366729.0	101.22			
3	364915	365312	365113.5	100.78			
4	367384	367448	367416.0	101.41			
5	365826	367549	366687.5	101.21			
6	367759	365501	366630.0	101.20			

Table 5: Sildenafil precision Result

S.No	Area-1	Area-2	Mean	% Assay	Mean	SD	%RSD
1	444957	444412	444684.5	101.29	100.97	0.20	0.19
2	443192	443103	443147.5	100.94			
3	442326	442812	442569.0	100.81			
4	444425	443023	443724.0	101.07			
5	442153	442447	442300.0	100.75			
6	444072	441991	443031.5	100.92			

Accuracy:

The accuracy was performed in triplicate by spiking the Sildenafil and tadalafil into the placebo mixture at 10%.25%, 50%, 100%, 200% and 300% of test concentration; from the area recovery values are calculated. The average recovery values are obtained within 98 to 102% shows that the method was accurate as per the ICH limits.

Table 6: Sildenafil accuracy results

%Spike level	wt of API spiked	Amount added	Amount recovered	%Recovery	Mean % recovery	%RSD
10	5.12	5.120	5.076	99.1	99.0	0.76
	5.03	5.030	5.017	99.7		
	5.11	5.110	5.020	98.2		
25	12.51	12.510	12.501	99.9	99.8	0.23
	12.53	12.530	12.473	99.5		
	12.50	12.500	12.496	100.0		
50	25.2	25.200	25.040	99.4	99.5	0.27
	25.1	25.100	24.947	99.4		
	25.1	25.100	25.059	99.8		
100	50.2	50.200	49.997	99.6	99.6	0.17
	50.1	50.100	49.960	99.7		
	50.2	50.200	49.889	99.4		
200	100.1	100.100	101.132	101.0	100.7	0.33
	100.2	100.200	100.621	100.4		
	100.2	100.200	100.704	100.5		
300	150.2	150.200	151.897	101.1	100.6	0.46
	150.4	150.400	150.862	100.3		
	150.1	150.100	150.626	100.4		

Table 7: Tadalafil accuracy results

%Spike level	wt of API spiked	Amount added	Amount recovered	%Recovery	Mean % recovery	%RSD
10	5.1	5.100	5.084	99.7	99.1	0.48
	5.1	5.100	5.047	99.0		
	5.1	5.100	5.039	98.8		
25	12.5	12.500	12.523	100.2	100.4	0.66
	12.5	12.500	12.477	99.8		
	12.40	12.400	12.538	101.1		
50	25.1	25.100	25.069	99.9	100.0	0.10
	25	25.000	25.016	100.1		
	25.1	25.100	25.080	99.9		
100	50.1	50.100	50.007	99.8	99.7	0.31
	50	50.000	49.954	99.9		
	50.2	50.200	49.862	99.3		
200	100.2	100.200	99.651	99.5	99.2	0.27
	100	100.000	99.147	99.1		
	100.3	100.300	99.214	98.9		
300	150.1	150.100	149.355	99.5	99.2	0.25
	150	150.000	148.737	99.2		
	150.2	150.200	148.727	99.0		

Solution stability:

The first three solutions prepared in the 2.5.2 section were checked for Sildenafil and tadalafil content at 24 and 48 hours by keeping it in closed container at room temperature, the variation from the initial value were within 2.0 % shows the solution was stable for 48 hours.

Table 8: Sildenafil solution stability compilation

No.	As such value	24 hrs Area(Bench Top)	% Assay	Difference
1	101.3	445561	102.23	-0.93
2	100.9	445130	102.13	-1.23
No.	As such value	48 hrs Area(Bench Top)	% Assay	Difference
1	101.3	446033	102.31	-1.01
2	100.9	443912	101.82	-0.92

Table 9: Tadalafil solution stability compilation

No.	As such value	24 hrs Area (Bench Top)	% Assay	Difference
1	101.6	370103	101.53	0.07
2	101.2	368676	101.14	0.06
No.	As such value	48 hrs Area	% Assay	Difference
1	101.6	369823	101.55	0.05
2	101.2	367092	100.80	0.40

Robustness:

To check the effect of deliberate changes in the method, the variation inflow rate (±0.05 mL) and variation in temperature (± 5ºC) are studied; result shows no effect on the method.

Table 10: Robustness results for sildenafil and tadalafil

Sildenafil						
S.No	**Parameters**	**Changed to**	**% RSD of standard**	**USP Tailing**	**USP Plate count**	**Resolution**
1	Flow rate	0.35 mL/min	0.30	1.11	170663	13.8
		0.45 mL/min	0.40	1.10	128964	15.5
2	Temperature	40ºC	0.2	1.1	145590	12.2
		50ºC	0.20	1.10	151212	17.5
Tadalafil						
1	Flow rate	0.35 mL/min	0.30	1.10	73665	13.8
		0.45 mL/min	0.40	1.10	74340	15.5
2	Temperature	40ºC	0.2	1.1	71732	12.2
		50ºC	0.30	1.10	73933	17.5

Ruggedness:

From the stock solutions of 3.1.3 the solution are prepared by filtering through PVDF PTFE 0.45 µm filter papers and the content of Sildenafil and tadalafil was tested. The results obtained are within 2%,shows there was no effect of various filters.

Table 11: Filter validation results of sildenafil

	Centrifuged	**PVDF, 0.45µm**		**PTFE,0.45µm**	
Test-1	100	100.50	-0.50	100.80	-0.80
Test-2	101.7	100.70	1.00	100.40	1.30

Table12: Filter validation results of tadalafil

	Centrifuged	**PVDF,0.45µm**		**PTFE,0.45µm**	
Test-1	100.7	101.20	-0.50	101.50	-0.80
Test-2	102.2	101.30	0.90	101.00	1.20

Intermediate precision:

Assay was performed by another analyst on different day, different system, and different column; the variation between the two analysts was less that 0.6 %, it shows that method was reproducible.

Table 13: Intermediate precision results for sildenafil

Sildenafil Preparation	**Analyst-1**	**Analyst-2**
	Assay values	**Assay values**
1	101.29	101.59
2	100.94	101.09
3	100.81	101.27
4	101.07	101.15
5	100.75	101.53
6	100.92	101.51
Average	100.97	101.36
Difference	-0.39	

Table 14: Intermediate precision results for Tadalafil

Tadalafil Preparation	Analyst-1 Assay values	Analyst-2 Assay values
1	101.62	102.3
2	101.22	101.6
3	100.78	101.6
4	101.41	101.5
5	101.21	102.2
6	101.20	101.5
Average	101.24	101.78
Difference	-0.54	

Study of un eluted peak:

The study shows that no peak eluted, proved that there was no un eluted peak with the developed method.

Forced degradation studies:

To prove the stability indicating power of the method the forced degradation studies are carried out. The degradation reagents were (30 mL) added after the disintegration kept on reflux for the specified time. In each condition the individual % of impurities and total impurities and assay are calculated. The mass balance obtained from the experiment was ranged 99 to 100%. In all the forced degradation conditions peak purity of Sildenafil, tadalafil and major degradants peaks are passed, it shows that the developed method was stability indicating.

Acid degradation:

It was performed with IN Hydrochloric acid for 48 hours and the degradation observed was 1.94% and the peak purity of sildenafil and tadalafil was passed, this proves that the method was stability indicating in acidic condition.

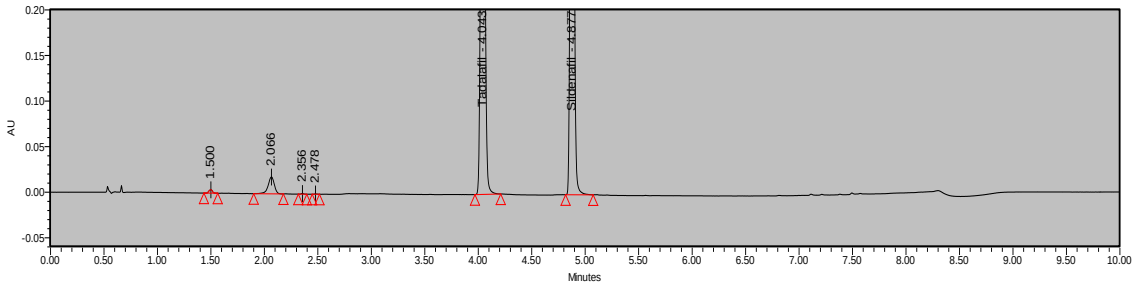

Figure 8: Acid degraded sample chromatogram

Base degradation:

It was performed with IN sodium hydroxide for 24 hours and the degradation observed was 0.12% and the peak purity of Sildenafil, tadalafil was passed, this proves that the method was stability indicating in Base degradation.

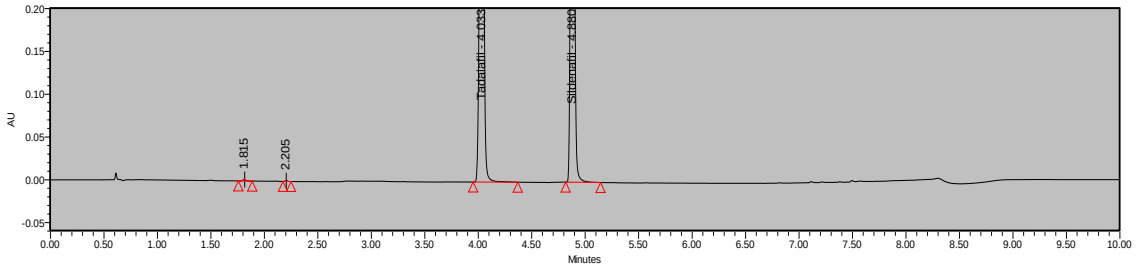

Figure 9: Base stressed sample chromatogram

Peroxide degradation:

It was performed with 10% Hydrogen peroxide for 24 hours and the degradation observed was 11.16 % the peak purity of sildenafil and tadalafil was passed, this proves that the method was stability indicating in Peroxide degradation.

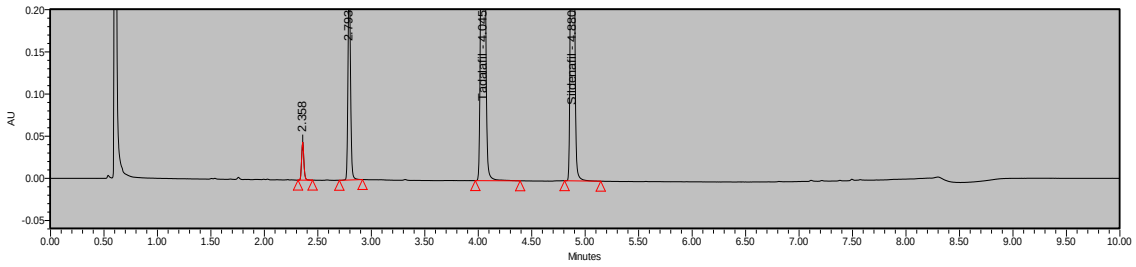

Figure 10: Peroxide stressed sample chromatogram

Water degradation:

It was performed with Milli Q water for 24 hours and the degradation observed was 0% and the peak purity of sildenafil, tadalafil was passed, this proves that the method was stability indicating in Water degradation.

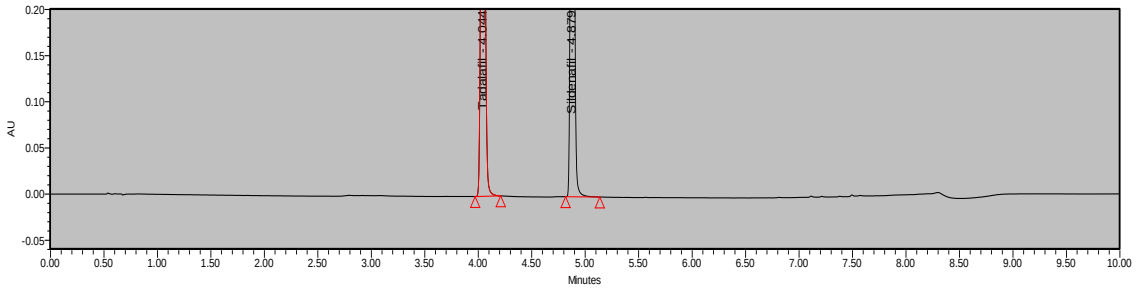

Figure 11: Water stressed sample chromatogram

Thermal degradation:

It was performed at 50ºC water for 48 hours and the degradation observed was 0% and the peak purity of sildenafil and tadalafil was passed, this proves that the method was stability indicating in Thermal degradation.

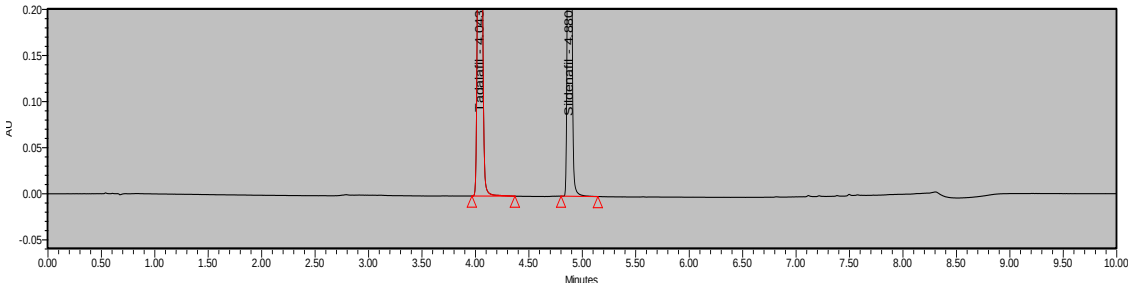

Figure 12: Thermal stressed sample chromatogram

Photo degradation:

It was performed till 1.2 million Lux hours visible light and 200 Watts UV exposure, the degradation observed was 0% the peak purity of sildenafil and tadalafil was passed, this proves that the method was stability indicating in Photo degradation.

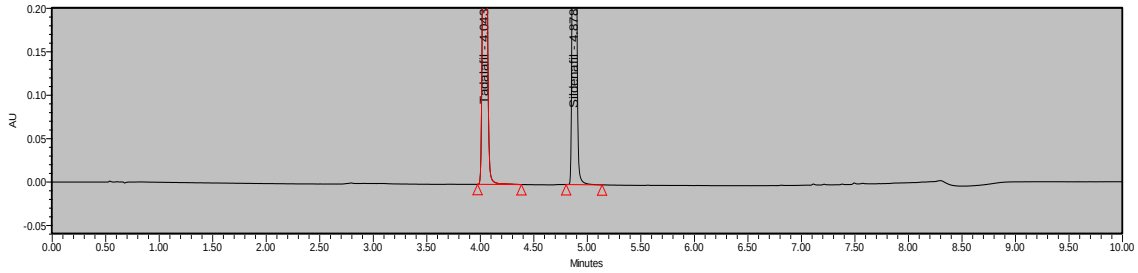

Figure 13: Light stressed sample chromatogram.

The overall summery of forced degradation results was as below.

Table 15: Forced degradation study results compilation

Type	Condition &Duration	% Degradation	Peak purity
Acid	1N Hcl,24 hours,50°C	1.94	Passes
Base	1N NaoH,1 day,50°C	0.12	Passes
Peroxide	10% H_2O_2,1 day,50°C	11.16	Passes
Water	Water,1 day,50°C	0%	Passes
Thermal	1 days,50°C	0%	Passes
Photo	1.2 million Lux hours	0%	Passes

System suitability equivalence:

The difference in the results between developed method and Pharmacopeia was much lesser. The critical pair resolution between sildenafil and tadalafil was maintained above 2.0 in the developed method, it shows that the results are comparable to that of Pharmacopeia method results.

Table 16: System suitability equivalence table for sildenafil peak

Parameter	Pharmacopeia method	UPLC Method
Assay Standard %RSD	0.2	0.3%
USP Plate count	6576	17034
USP Tailing	1.02	1.04

Table 17: System suitability equivalence table for tadalafil peak

Parameter	Pharmacopeia method	UPLC Method
Assay Standard %RSD	0.3	0.4
USP Plate count	7748	12561
USP Tailing	1.02	1.02

API Batch analysis results equivalence:

The results obtained with the developed method was compared with the API Vendor method results, the variation in assay and impurities results was below 0.1%, proves that the method was equivalent to the API Vendor method with 10 minutes runtime.

Table 18: API Analysis results equivalence table for sildenafil API

Details	Pharmacopeia method	UPLC Method results
B.No:SC0091011 Assay	99.7%	99.6%

Table 19: API Analysis results equivalence table for sildenafil API

Details	Pharmacopeia method	UPLC Method results
B.No:TDM0020212 Assay	99.4%	99.5%

Reference product analysis results equivalence:

The results obtained with the developed method shows the difference less than 0.5% with Pharmacopeia method, shows that the developed method is equivalent to that of Pharmacopeia method with 10 minutes Run time.

Table 20: Reference product analysis (Viagra) equivalence table

Details	Pharmacopeia method	UPLC Method results
B.No:A068901 Assay	100.1%	100.0%

Table 21: Reference product analysis (cialis) equivalence table

Details	Pharmacopeia method	UPLC Method results
B.No:A893165 Assay	100.3%	100.2%

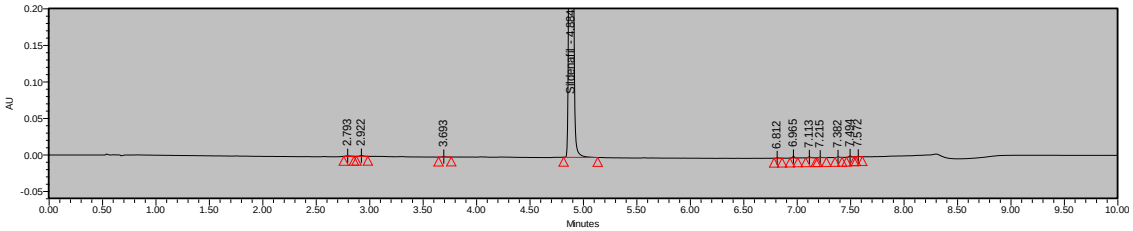

Figure 14: Viagra sample chromatogram.

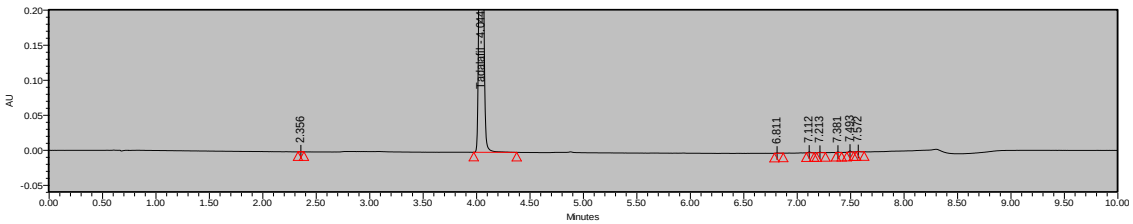

Figure 15 : Cialis sample chromatogram.

CONCLUSION:

An UPLC method for related compounds in the commercial drug products and in the tablet formulation was validated in this study. Sildenafil, tadalafil, their degradants and impurities gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %, the slope and the intercept, Solution stability) calculated were within the acceptable limits and shown equivalent to the Pharmacopeia methods. The method can be used for estimation of sildenafil and tadalafil in bulk drugs and its tablet dosage forms for quality control purposes.

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