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Reverse phase – High performance liquid chromatographic method for simultaneous estimation of sildenafil citrate and tadalafil in tablets dosage form

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ABSTRACT

An isocratic liquid chromatographic method is described for simultaneous determination of Sildenafil citrate and Tadalafil in Tablets dosage forms. Chromatographic separations of both drugs was achieved on a Purosphere Star C-8 column (150mm X4.6mm, i.d., 5µm,) using a mobile phase consisting of Water: Acetonitrile: Trifluoroacetic acid (65:35:0.1% v/v), with flow rate of 1.0ml/min and detection, was carried out at 220nm and 20ul injection volume was selected. The optimum separation was achieved in less than 15 minutes. The developed Liquid Chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. The developed method obeys beer's law over the concentration range of 25 to 75µg/ml for Sildenafil and 5 to 15µg/ml for Tadalafil. The method was validated as per ICH guidelines and is suitable for the routine analysis of Sildenafil citrate and Tadalafil in tablet dosage forms.

Keywords: *RP-HPLC sildenafil and tadalafil, Sildalis tablets simultaneous.*

1. INTRODUCTION

The chemical name of sildenafil (SLD) is 5-(2-Ethoxy-5-[(4-methyl-1-piperazinyl)sulfonyl]phenyl)-1-methyl-3-propyl-1,4-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one 2-hydroxy-1,2,3-propanetricarboxylate (1:1). Its empirical formula is C28H38N6O11S, White to off-white powder and its molecular weight 666.703gm/mol in Figure 1. The chemical name of Tadalafil (TDL) is (6R-trans)-6-(1,3-Benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[(1,2:1,6] pyrido[3,4-b]indole-1,4-dione and its empirical formula is C22H19N3O4 and White to off-white powder in Figure 2.

Sildenafil and Tadalafil are phosphodiester type 5 (PDE5) inhibitors used to treat the erectile dysfunction (ED) by increasing the levels of cGMP1.

The literature survey reveals various HPLC methods were developed for individual components. Only one method (Ref No. 1) was developed for this combination but restricted to bulk drugs only. Hence, an attempt has been made to develop HPLC method confirms for the determination of Sildenafil and Tadalafil in pharmaceutical Tablets dosage forms. The method was optimized and validated according to International Conference on Harmonization (ICH). The present study was aimed to develop a simultaneous, simple, rapid, precise, accurate, stable and selective reversed phase chromatographic method to estimate the Sildenafil and Tadalafil in pharmaceutical Tablets dosage forms.

2. EXPERIMENTAL METHODOLOGY

Working standards of Sildenafil and Tadalafil procured from CDTL –Mumbai with defined potency was used. SILDALIS film-coated tablets containing Sildenafil 100 mg and Tadalafil 20 mg (Export quality) and Placebo for examination were procured from Paralab, Mumbai. HPLC grade Acetonitrile, GR grade Trifluoroacetic acid and milli-Q-grade water were used for diluents and mobile phase.

Instrumentation: Waters Alliance HPLC system equipped with PDA detector and EMPOWER 2 software was used to monitor the data acquisitions and other proceedings. Purosphere Star C-8 column (150mm X 4.6mm, i.d., 5µ) was used as stationary phase.

Selection of Diluent: - Considering chemical nature of both drugs. Water: Acetonitirle (1: 1) was selected for preparation of Sample and Standard.

Determination of wavelength of maximum absorbance: The standard solutions of Sildenafil (20ug/ml) and Tadalafil (5ug/ml) were scanned in the range of 200 -400 nm against diluent as a blank. Both Sildenafil and Tadalafil have shown maximum absorbance at 220nm. So the wavelength selected for the determination of Sildenafil and Tadalafil was 220 nm in Figure 3 & 4.

Mobile phase: - Mobile phase consisting of Water: Acetonitrile: Trifluoroacetic acid

(65:35:0.1% v/v)

Preparation of Standard solution: a Standard solution containing 50µg/ml of Sildenafil citrate and 10µg/ml of Tadalafil was prepared in diluents.

Preparation of Sample solution: A composite of 20 Tablets was prepared by grinding them to a fine, uniform size powder for preparing the sample. Sample equivalent to 20 mg of Tadalafil was accurately weighed and quantitatively transferred into a 200 ml volumetric flask. Approximately 100 ml diluent was added and the solution was sonicated for 15 min. The flask was filled to volume with a diluent, and mixed. After filtration, an amount of the solution was diluted with diluent to get a concentration of 50µg/ml of Sildenafil and 10µg/ml of Tadalafil.

Chromatographic Conditions: - Purosphere Star C-8 column (150mm X4.6mm, i.d., 5µm,) using a mobile phase consisting of Water: Acetonitrile: Trifluoroacetic acid (65:35:0.1% v/v), with flow rate of 1.0 ml/min and detection was carried out at 220nm and 20ul injection volume was selected.

Method Optimization:- The chromatographic conditions were optimized by taking into consideration the chemical structures of Sildenafil citrate and Tadalafil (Figure 1 & 2), choice of the column with respect to chemistry of packing material, dimension of column, the composition, pH, flow rate of mobile phase, the wave length of detection and injection volume. Purosphere Star C-8 column (150mm X4.6mm, i.d., 5µm,) was selected for method development. Solubility data show that both drugs are sparingly soluble in methanol, insoluble in water, soluble in weak acids. Hence Acetonitrile and water were taken the first choice as the mobile phase for the retention of both drugs. Different proportions of water and Acetonitrile were employed to achieve the separation between these two compounds and separation was obtained in the ratio of (65:35) of water and Acetonitrile, however high tailing factors (about 2.26 for both drugs) was observed which is the unusual limit. In order to improve the peak shape different organic modifiers were used out of which 0.1% trifluoroacetic acid (pH about 2.0) was found to be more suitable for the separation of both drugs.

Percent Purity & Percent Recovery: For the determination of percent purity of analytical method, approx. 50ug/ml of Sildenafil and 10ug/ml of Tadalafil concentration of market formulation was injected and observed concentration of market formulation was 99.67 mg/tab of Sildenafil and 19.99mg/tablets and %purity was obtained 99.67% and 99.93% respectively in Table 1.

3. METHOD VALIDATION

Method validation was performed following ICH specifications for specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

Specificity: The results of the specificity done by injecting Placebo and Blank (Diluents) & was found that there was no interference and co-elution of any other peaks with the Sildenafil and Tadalafil. The peak purity of Sildenafil and Tadalafil standard and sample of Tablets dosage form found within the limit which proved that there was no interference between the blank and placebo peaks in **Figure 5**.

Linearity: Several aliquots of standard stock solutions of Sildenafil and Tadalafil were taken in different 50ml volumetric flasks and diluted up to the mark with a diluent such that the final concentration of SLD and TDL is 25-75µg/ml and 5-15µg/ml respectively in **Table 2**

The linear calibration plot was constructed by analyzing the concentrations over the selected range and the sample volume of 20 µl was injected three times into the column and it was used for the determination of peak area for both drugs. The correlation coefficient obtained from the linearity studies showed a good linear response with limits of correlation between the peak area and concentration of the analytes in **Figure 4 & 5**.

Precision: The intra-day and inter-day precision were determined by injecting the six replicates of standard concentration into the HPLC system. The % RSD was calculated from the peak area responses of the concentration on the same day and it on consecutive days (n=3). The result showed the % RSD was found to be less than 2.0 and it indicates the precise method. The summarized results were shown in **Table 3**.

Accuracy: For studying the accuracy of the proposed analytical method and for checking the interference from excipients used in dosage forms, recovery experiments were carried out by spiking standard with Placebo. The Standard concentration with levels of 80%, 100% and 120% to the placebo. The known quantity of Tadalafil and Sildenafil standard concentration was spiked with placebo and results showed good recovery in **Table 5**.

Limit of Quantification: The LOQ were evaluated on the basis of the linearity curve. LOQ of Sildenafil and Tadalafil were found to be 50µg/ml and 10µg/ml respectively. The results showed that the method can be efficiently applied for the estimation of Tadalafil and Sildenafil.

Robustness: The robustness was conducted by a deliberate change in the optimized chromatographic conditions. The mobile phase variation (± 3% v/v), flow rate (±0.2 ml/min), wave length (±2 nm), column oven temperature (±5 ºC), were slightly changed. The reproducible results were obtained which proves the robust method.

Stability in solution: The stability of Tadalafil and Sildenafil in a solution containing mobile phase with placebo have been determined by keeping one sample in the refrigerator and other in a tightly capped volumetric flask placed at ambient temperature under normal lighting conditions. The samples were checked for assay on two successive days of storage and compared with the freshly prepared sample. The RSD values of experiments were found to be below 2.0% in both cases. This indicates that the Tadalafil and Sildenafil are stable in the solution.

4. RESULTS AND DISCUSSION

Novel and simple HPLC method have been developed for the simultaneous determination of Sildenafil and Tadalafil in Tablet dosage forms. Only the mixture of water, Acetonitrile, and Trifluoroacetic acid in the ratio of 65:35: 0.1% v/v gave excellent resolution, whereas any other ratio gave poor resolution and close retention times between Sildenafil and Tadalafil. The retention times were 3.8 (T 1.1, %RSD: 0.08) for Sildenafil and 10.4(T: 1.06, %RSD: 0.11%) for Tadalafil. System suitability study results show that the method is precise and accurate. The SST experiment is applied to a representative chromatogram and results obtained are shown in Table 7.0 that is concurrence with the USP requirement. The linearity study shows that linear regression found to be more than 0.999 for both components i.e. Sildenafil and Tadalafil in **Figure 4 & 5**.
Limit of detection was established by signal to noise ratio method.The LOD for Sildenafil and Tadalafil were found to be 0.025 and 0.05 µg/ml whereas LOQ for Sildenafil and Tadalafil were found to be 50ug/ml and 10µg/ml respectively. Percentage recoveries for both drugs were found to be near to 100% show that the method is free from interferences due to the excipients. Good recoveries and low RSD values indicate the precision of the method**.**

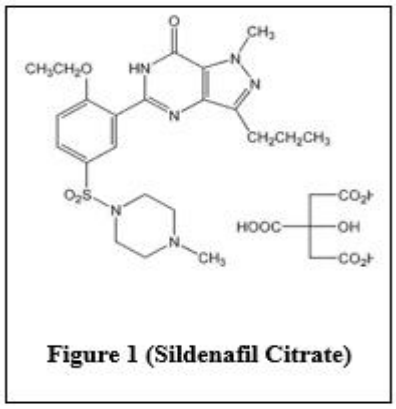

Figure 1 (Sildenafil Citrate)

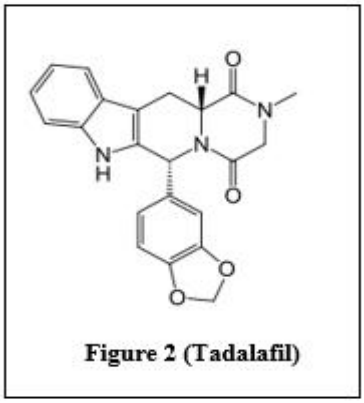

Figure 2 (Tadalafil)

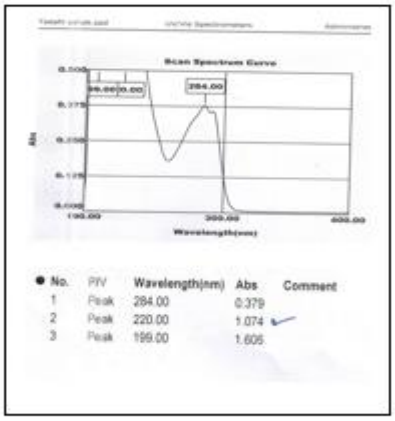

Figure 3: UV spectra of Sildenafil Citrate

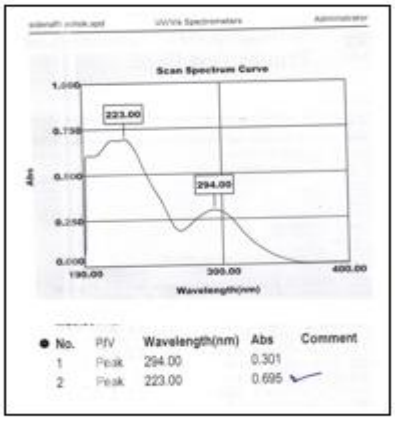

Figure 4: UV spectra of Tadalafil

Table 1

Drugs	Claim (mg/tablets)	Amount Found (mg/tablets)	% label claim
Sildenafil	100mg	99.67mg	99.67
Tadalafil	20mg	19.99mg	99.93

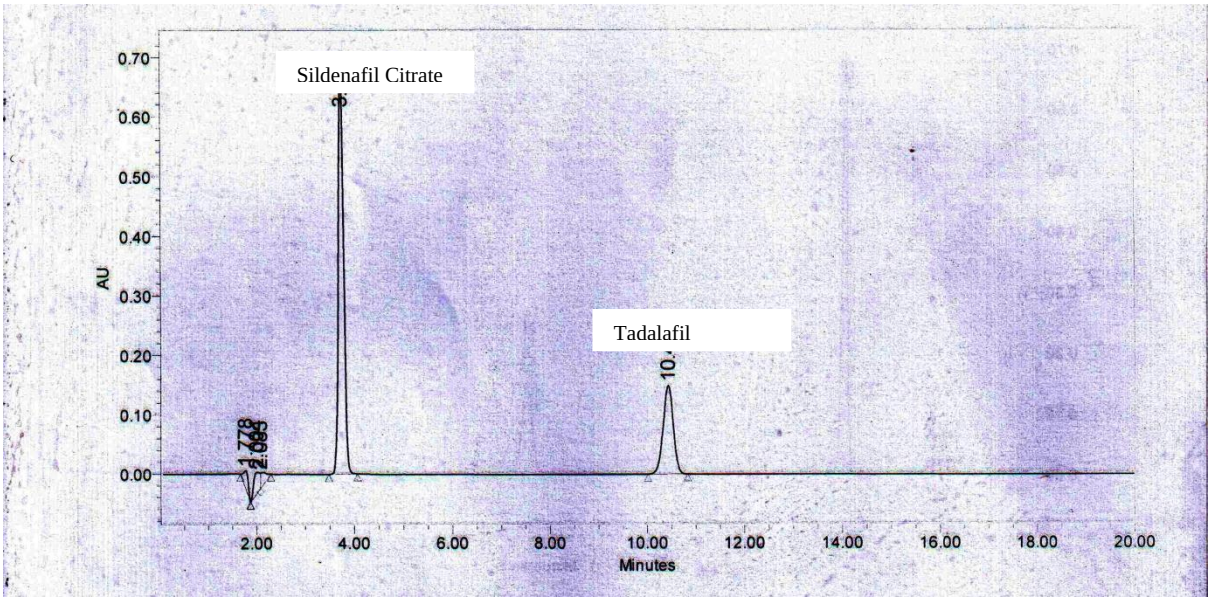

Figure 5: Chrmatogram of Sildenafil & Tadalafil in the marked formulation

Table 2: Linearity range of Sildenafil Citrate and Tadalafil

Conc (ug/ml)	Peak Area of SLD	Conc (ug/ml)	Peak Area of Tadalafil
5	136040	1	54617
15	616119	3	263851
25	1160396	5	496171
40	2006163	8	872262
50	2595828	10	1095136
60	3104454	12	1304247
75	3910136	15	1650403

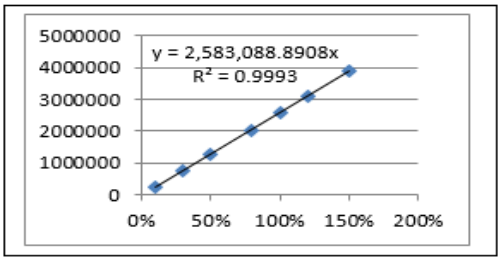

Figure 6: Linearity Curve of Sildenfil

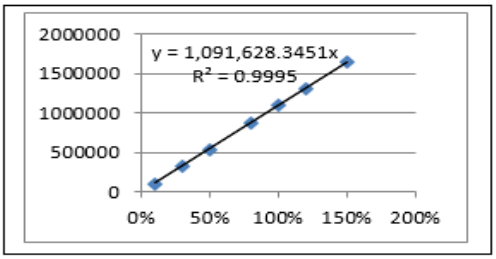

Figure 7: Linearity Curve of Tadalfil

Table 3: Precision of developed analytical method

Injection No.	Area	
	Sidenafil	Tadalafil
1	2592146	1092455
2	2591123	1093807
3	2592948	1090780
4	2596847	1091277
5	2593985	1091146
6	2593142	1091110
Mean	2593410	1091893
± SD	2190	1241
% RSD	0.08	0.11
LimitNMT	2%	2%

Table 5: Accuracy of the developed analytical method

Sr. No.	Conc.	Accuracy	
1	80%	98.69	99.67
2	100%	98.53	99.62
3	120%	98.29	99.31

Table 4: System Suitability Parameters

System Suitability Parameters	Sildenafil	Tadalafil
Retention time	3.73 min	10.43 min
Theoretical plates (N)	7255	12097
Tailing factor	1.1	1.06
Resolution		19.4

5. CONCLUSION

An analytical method was developed and validated for the determination of Sildenafil citrate and Tadalafil in pharmaceutical tablets dosage forms. Analysis data indicates that the developed method is simple, selective and specific for estimation of Sildenafil citrate and Tadalafil in tablets pharmaceutical formulations.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

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