A SIMPLE AND SENSITIVE REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF TADALAFIL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of Tadalafil in bulk and in Pharmaceutical formulation. The quantification was carried out using Cyberlab capcell pak, ODS C_{18} (250 × 4.6mm i.d., 5µm particle size) column in an isocratic mode, with mobile phase comprising buffer KH_2PO_4 adjusted to pH 6.8 with orthophosphoric acid: acetonitrile: methanol in the ratio of 60:30:10 (%v/v/v). The flow rate was at 1.2 ml/min and the detection was carried out at 284 nm. The retention time of the drug was found to be 7.89 min and the method produced linear response in the concentration range of 10-100 µg/mL (r~0.9999). The recovery studies were also carried out and % RSD from reproducibility was found to be 0.627. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Tadalafil in tablets.

KEY WORDS: RP-HPLC, Tadalafil, Tablets.

1.INTRODUCTION

Tadalafil (Figure1), chemically it is a pyrazino [12 ,22 :1,6] pyrido [3,4-b] indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12 a-hexahydro-2-methyl (6R,12aR) . It is an impotence agent. It is indicated for the treatment of erectile dysfunction (Pomerol and Rabasseda,2003; Seftel,2004). It is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) (Francis and Corbin,2003). Tadalafil has the empirical formula of $C_{22}H_{19}N_3O_4$ and representing a molecular weight of 389.41. It is a crystalline solid that is practically insoluble in water and very slightly soluble in ethanol and soluble in methanol.

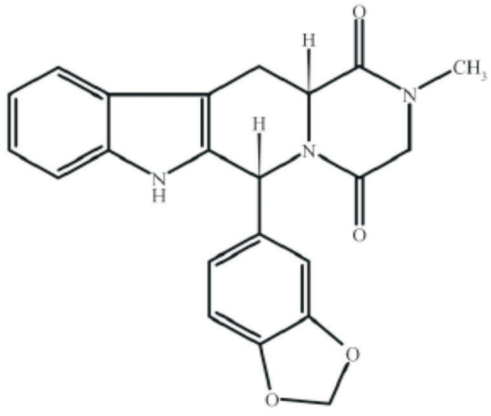

Figure 1. Structure of Tadalafil

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Literature survey reveals that very few analytical methods have been reported for the determination of Tadalafil in pharmaceuticals and biological fluids. Tadalafil was determined in human serum and urine by LC-MS/MS (Ramakrishna,2004; Zhu,2005) and HPLC with UV detection (Cheng and Chou,2005) and also by micellar electrokinetic capillary chromatography (Rodriguez,2004) and densitometric TLC (Berniati,2006) methods has also been reported.

The objective of this study is to develop a simple, fast, selective, accurate, precise and sensitive RP-HPLC-UV method for the determination of Tadalafil in bulk and in Pharmaceutical dosage forms (tablets) suitable for routine quality control analysis.

2.MATERIAL AND METHODS

Materials

Tadalafil working standard was received as gift sample from Orchid chemicals and Pharmaceuticals ltd., Chennai, India. A Tadil-10 mg tablet, manufactured by Ind-Swift Ltd., was procured from local Pharmacy. HPLC grade Water, Methanol and Acetonitrile were purchased from Merck, Mumbai, India. Potassium dihydrogen phosphate and ortho phosphoric acid were purchased from S.d.Fine chemicals ltd., Mumbai.

Instruments and Chromatographic conditions

The method development study was carried out isocratically on a high performance liquid chromatograph

using Cyber lab LC-100 separation module equipped with a Rheodyne injector 7725 i, Single pump, 20µL fixed sample loop, 25µL Hamilton syringe and detection was carried out using Ultraviolet detector.Cyberlab Digital balance was used for weighing purpose.

Chromatographic separation was carried out at room temperature with Capcell Pak ODS C_{18} (250 × 4.6 mm with 5 µm particles) column. Mobile phase containing Buffer (KH_2PO_4 adjusted to pH 6.8 with ortho phosphoric acid: acetonitrile: methanol in the ratio of 60:30:10 %v/v/v), were filtered through 0.45 µ membrane filter and degassed in a sonicator for 15 min before use. The flow rate of mobile phase was maintained at 1.2 ml/min and detection was done using UV detector at 284 nm.The injection volume of both standards and samples were 10 µL (100 µg/mL).

Method:

Preparation of standard

A stock solution containing 1 mg/ml of Tadalafil was prepared by completely dissolving 100 mg of pure drug of Tadalafil in 100 ml of methanol. A working standard solution containing 100 µg/ml was prepared by diluting 5 ml of stock solution (1000 µg/ml) into 50 ml of mobile phase. Linearity solutions ranging 10-100 µg/ml of Tadalafil were prepared from the above working standard solution (100 µg/ml) by diluting into a 10 ml volumetric flask with the mobile phase. Initially the mobile phase was pumped for 30 min to saturate the column there by to get the baseline corrected as shown in Fig.2.Then solutions prepared as above were filtered through 0.45 µ membrane filter and then 10 µL of the filtrate was injected each time into the column at a flow rate of 1.2 mL/min. Evaluation of the drug was performed with UV-Visible detector at 284 nm after the drug solution of 10 µg/ml in methanol was scanned in UV-Visible spectrophotometer SL-164 in the range of 200-350 nm against methanol as blank and found ë $_{max}$ at 284 nm as show in Fig.3. Peak area was recorded for all the peaks. The plot of peak area versus the respective drug concentration gives the calibration curve. The retention time of Tadalafil standard was found to be 7.89 minutes as shown in Fig.4

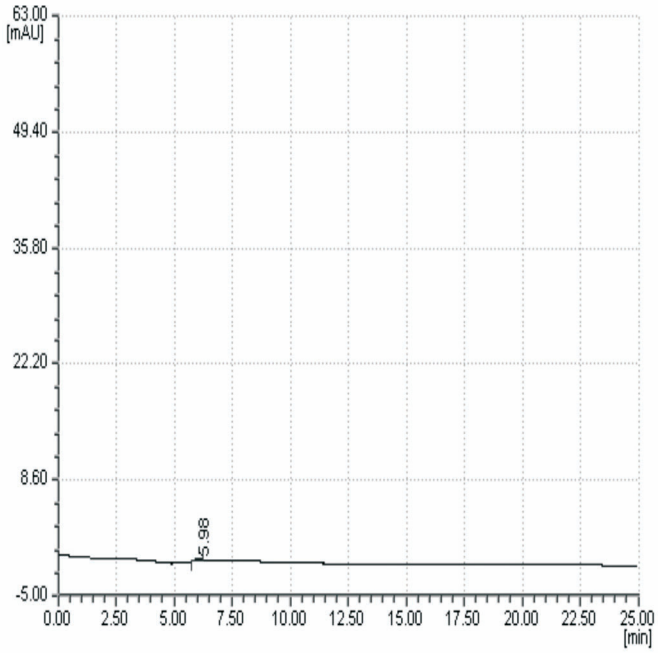

Figure 2.Chromatogram of Tadalafil blank

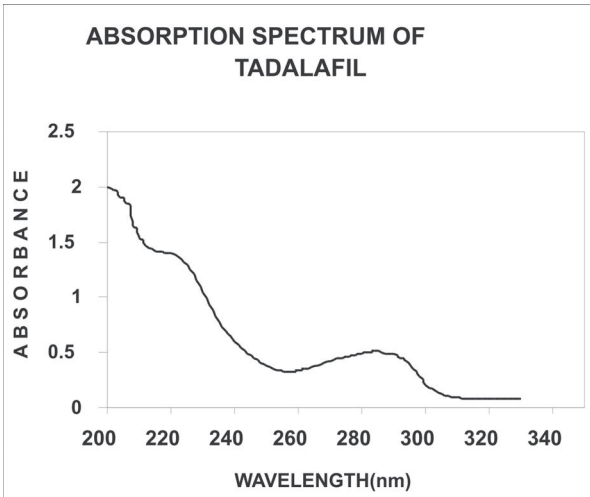

Figure 3.Absorption spectrum of Tadalafil (10 µg/ml) in methanol

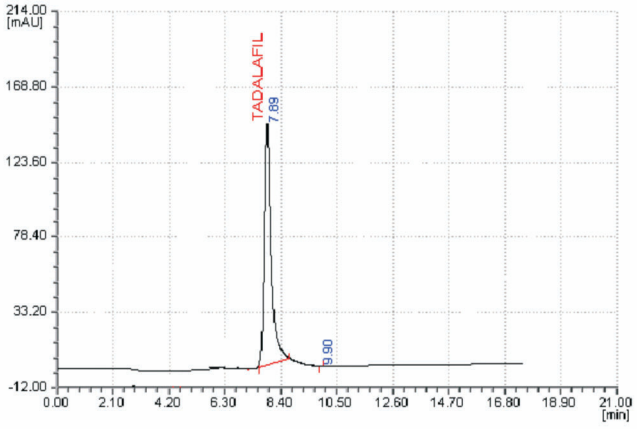

Figure 4.Chromatogram of Tadalafil standard

Analysis of Tadalafil in tablet dosage forms

Twenty tablets each containing 10 mg Tadalafil were accurately weighed and powdered. A quantity of the powder equivalent to 100 mg was taken into a 100 mL volumetric flask and 70 mL methanol was added. Then solution was sonicated for 10 mins, dissolved and then made upto the volume with the methanol and filtered through a 0.45 µ membrane filter. Then 10 ml of the above filtrate was transferred into a 100 ml volumetric flask and diluted to the mark with the mobile phase to obtain working standard solution of 100 µg/ml. Then 10 µL of the above solutions were injected each time into the column at a flow rate of 1.2 mL/min. The retention time of Tadalafil samples were found to be 7.87 and 7.98 minutes as shown in Fig.5 and Fig.6.

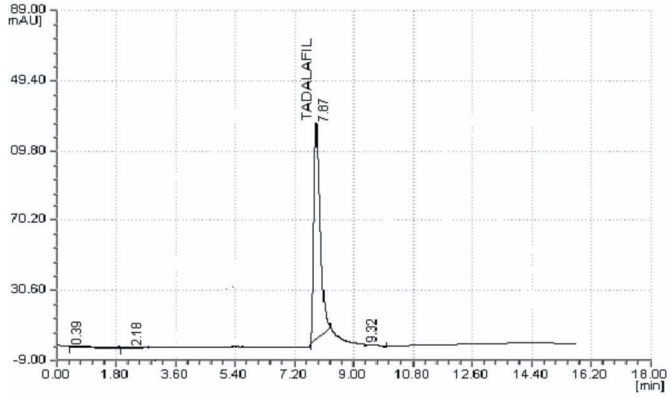

Figure 5.Chromatogram of Tadalafil sample 1 (in tablet)

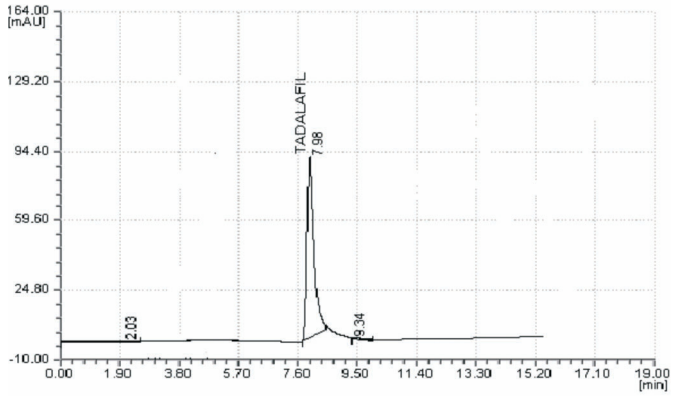

Fig.6.Chromatogram of Tadalafil sample 2 (in tablet)

3.RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise RP-HPLC (Reverse phase high performance liquid chromatographic) method for the analysis of Tadalafil in bulk and in tablet dosage forms. For the determination of Tadalafil, different compositions of mobile phases were employed. Initially, a mobile phase consisting of acetonitrile and water in the ratio of 60:40 %v/v and methanol and water in the ratio of 80:20 %v/v were tried where broad peak shapes and more retention times were observed. Then the composition of mobile phase was changed to Phosphate buffer adjusted to various pH conditions, acetonitrile: water in different ratios but in all these conditions more retention time and tailing were observed but finally the ratio was changed to 60:30:10 %v/v/v of Phosphate buffer: acetonitrile: methanol, where Tadalafil was eluted at around 7.89 min with symmetric peak shape and shorter retention time. The results of system suitability parameters were given in the Table-1.

Table-1. Results of System Suitability Parameters of Tadalafil in standard and in tablet formulation

S.No.	Parameter	Standard	Sample 1	Sample 2
1	Retention Time (Min)	7.89	7.87	7.98
2	Peak area response	268235.2	267257.5	267949.1
3	Theoretical plates(n)	6105.8	6220.5	5399.8
4	Tailing factor(t)	1.80	1.79	1.89

Linearity was determined from calibration graph plotted using peak area response versus concentration of the standard solutions and it was found to be obeyed in the concentration range of 10-100 µg/ml with a good linear relationship (r=0.9999) as shown in Fig.7.The regression curve was constructed by linear regression fitting and its mathematical expression was y=2691.93 x -1328.4 (where y is the peak area and x is the concentration of Tadalafil).

Precision of the developed method was studied by repeatedly injecting Tadalafil standard and sample solutions for six times (n=6). The % RSD was found to be 0.627 and 0.842 respectively.

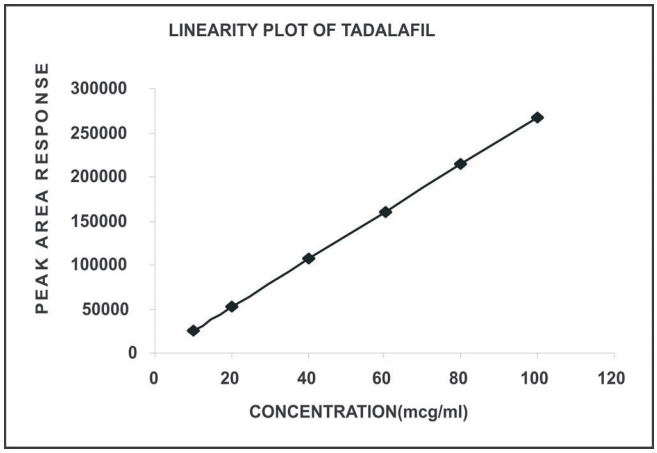

Figure 7. Linearity Graph of Tadalafil.

The drug content (Assay) in the tablets was quantified using the proposed RP-HPLC method. The mean amount of Tadalafil in single brand of tablet dosage form is shown in Table-2. The tablet was found to contain 99.64 % and 99.89 % of the drug. It can be concluded that the proposed RP-HPLC method is sufficiently sensitive and reproducible for the analysis of Tadalafil in tablet dosage form within a short analysis time.

Table-2.Results of Assay in Marketed formulation

S.No.	Brand	Standard Peak Area	Sample Peak Area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay
1	TADIL	268235.2	267257.5	10	9.964	99.64
2	TADIL	268235.2	267949.1	10	9.989	99.89
				Mean	99.77	
				% RSD	0.176	

The accuracy of the method was evaluated by performing recovery studies by analyzing three different concentration levels ranging from 50-150% of the test concentrations. The percentage recovery was calculated and results are presented in Table-3.

Table-3.Results of Accuracy (Recovery studies, n=3)

S.no.	Test concentration	Amount added (µg/ml)	Amount Recovered (µg/ml)	Average % Recovery	% RSD
1	50%	5	105.02	100.01	0.17
2	100%	10	109.97	99.97	0.42
3	150%	15	114.98	99.98	0.35

The developed method was validated according to the standard procedure and the summary of results obtained is presented in Table-4.

Table-4.Summary of Validation Parameters

S.no.	Parameters		Result
1	Linearity	Range (µg/ml)	10-100 µg/ml
		Correlation Coefficient (r)	0.9999
		Slope	2691.93
		Intercept	-1328.4
		Regression Equation	Y=2691.93 x-1328.4
2	System Precision (n=6)	%RSD	0.627
3	Method Precision (n=6)	%RSD	0.842
4	Accuracy	Mean % Average Recovery	99.98
5	Assay	Mean % Assay	99.77
6	Specificity	Specific	No interference of other peak

4.ACKNOWLEDGEMENT

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