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RP-HPLC-PDA method development and validation for the analysis of Tadalafil in bulk, pharmaceutical dosage forms and in-vitro dissolution samples

Aziz Unnisa, Yogesh Babu, Santosh kumar suggu, Siva Chaitanya

KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, AP, India.

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ABSTRACT

The present work describes the development and validation of a simple, rapid, selective and reproducible RP-HPLC method for the Analysis of Tadalafil in Bulk, tablets and *In-Vitro* Dissolution Samples. Analysis was performed on an Agilent, EclipseC18 column (150 mm × 4.6 mm, 5 μm) with the mobile phase consisting of ammonium acetate (10 mM):methanol (35:65v/v) at a flow rate of 1.0 mL/min. UV detection was performed at 280 nm and the retention time for TDL was 4.6 minutes. The dissolution media composed of 0.5% SLS, using USP II (Paddle) Dissolution apparatus, at 50 RPM and temperature maintained 37.0 ± 0.5°C. The method was validated according to ICH guidelines. The method was validated for specificity, accuracy, precision, ruggedness, limit of quantification, limit of detection and linearity. The system suitability parameters, such as , tailing factor, theoretical plate and relative standard deviation (RSD) for assay of five standard replicates, were well within the limits. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate. Hence the method can be used for the routine Dissolution Profiling of Tadalafil as well as its assay in bulk and tablets .

INTRODUCTION

Tadalafil [(TDL) Figure 1] is used to treat male erectile dysfunction. TDL belongs to phosphordieterase type-5 inhibitor (PDE5) class (Sweetman, 2002). It inhibits cGMP specific PDE5 which is responsible for degradation of cGMP in the corpus cavernosum located around the penis. In order to prevent the malpractices of Counterfeiting drugs that has now become a multi-billion dollar business that threatens the well being of the humanity and effective delivery of health care services (Abdulmumin, 2011) and to ensure quality of drugs; simple, sensitive and selective methods for their QC analysis are indispensible. Dissolution studies and Assays are routinely performed in Quality Control (QC) and R&D Laboratories for the evaluation of pharmaceutical products (Azarmi *et al.*, 2012).

The literature review showed a few HPLC methods for estimation of TDL individually (Sankar and Arulantony, 2013; Sonawane *et al.*, 2013;) or in combination with other drugs like Sildenafil citrate (Kannapan *et al.*, 2010) and Dapoxetine hydrochloride (Abha *et al.*, 2012 ; Bharat *et al.*, 2013), LC-MS (Xiaolan Zhu *et al.*, 2005), UV-Visible Spectrophotometry (Mohammad *et al.*, 2010, Safwan, 2014) and Flourimetry (Kavitha *et al.*, 2013) methods were reported so far. However there is no RP-HPLC estimation for dissolution profiling of TDL.

All the reported methods either took a long time for analysis or employed mobile phases with pH adjustment of buffer solutions which is tedious and not suitable, particularly for routine testing of tablet and dissolution samples.

Hence, an attempt was made to develop a rapid, simple, accurate and reproducible and LC-MS compatible RP-HPLC-PDA method for the estimation of TDL in tablet dosage forms and in dissolution samples to support product development and quality control efforts.

* *Corresponding Author*
Aziz Unnisa, KVSR Siddhartha College of pharmaceutical sciences Vijayawada, AP, India. Email: mail id: khushiazeez@yahoo.co.in

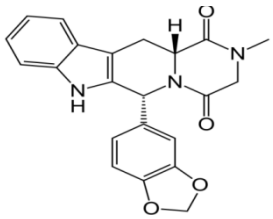

Fig. 1: Chemical structure of Tadalafil.

MATERIALS AND METHODS

Materials

TDL pure drug was gift sample obtain from Macleodes, India. HPLC Grade Ammonium Acetate and Methanol were procured from E. Merck Ltd, India. Sodium Lauryl Sulphate was purchased from Loba Chemie, Mumbai, India.0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. India. Mili-Q water was used throughout the experiment. Megalis-10 mgtablets were purchased from local pharmacy.

Instrumentation

Analysis was performed on a Shimadzu HPLC chromatographic system, class VP series (Japan) equipped with SIL-20A auto sampler, programmable variable wavelength PDA detector SPD-M20A VP and two LC-20AD pumps. Chromatographic separation was achieved by Agilent eclipse C_{18} column (150×4.6 mm, 5 µm). The HPLC system was equipped with "LC-Solution" software to acquire and process the data. Peak purity was checked with the PDA detector. All dissolution studies were carried out using Dissolution test apparatus (DISSO 8000, Lab India).

METHODOLOGY

Dissolution studies

In vitro dissolution studies of TDL tablets was carried out in 900mL of 0.5% SLS using USP type-II (paddle) Dissolution test apparatus. The tablet was directly placed in the dissolution medium. A speed of 50 rpm and a temperature of 37±0.5°C were used in the test. A 5mL aliquot was withdrawn at 10, 15, 20, 30 and 45 min and replaced with 5mL of fresh dissolution medium. The samples were filtered using 0.45µm nylon disc filter, suitably diluted if necessary and analysed by HPLC. The dissolution experiments were conducted in triplicate. The amount of TDL in the test samples was calculated as percentage drug dissolved, by comparing peak area of the test samples with standard.

Standard solutions and calibration plots

Accurately weighed amount of standard TDL bulk (10 mg) was transferred to a 10 mL volumetric flask containing few mL of MeOH, the solution was sonicated for 2min and volume was made up to the mark with the same solvent to obtain final concentration of 1mg/mL. Aliquots of above stock solution containing 5 to 25 µg of drug were transferred in to a series of centrifuge tubes and the volume was made up to 1 mL using ammonium acetate. 20 µL of standard at each concentration level was injected into the column in triplicate. The mean values of peak areas were plotted against concentrations.

Liquid chromatographic conditions

An isocratic HPLC analysis was performed on Agilent, EclipseC18 column (150 mm × 4.6 mm, 5μm)maintained at ambient conditions (37 ± 0.5^0 C).Chromatographic separation was achieved with the mobile phase ratio of 35:65 (v/v) mixture of 10 mM ammonium acetate and MeOH at flow rate of 1.0ml/min and injection volume of 20 µL. UV spectra of TDL (10 µg/mL) was scanned under these conditions and from the spectra a maxima of 280 nm was observed (Figure 2). For quantitative analytical purpose wavelength was set at 280 nm, which also provided a better reproducibility.

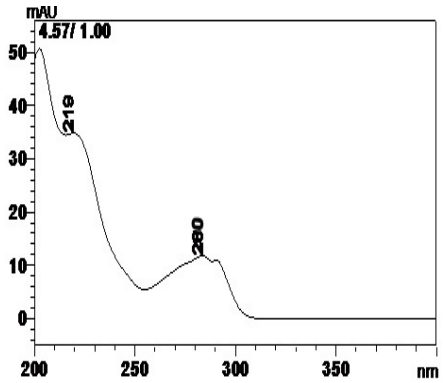

Fig.2: TDL UV absorbance spectra.

Method validation

Specificity

Placebo solution was prepared as that of the test solution using equivalent weight of the placebo in a portion. The diluent, placebo, pure drug and sample solution were injected into the HPLC system following the test conditions, the chromatograms were recorded for each of the solutions and the response of the peaks were noted for examining interference.

Linearity

The Linearity was established at the concentration range of 5-25 µg/mL for TDL. The standard stock solution (1000 µg/mL) was diluted with MeOH to get standard solutions in the range of 5-25 µg/mL TDL. Each concentration was analyzed in triplicate. Peak areas (y) of TDL were plotted versus their respective concentrations (x), linear regression analysis performed on the resultant calibration curves and correlation coefficient (R) was calculated.

System suitability

System suitability was carried out by injecting 10µg/mL of Tadalafil at different injection volumes ranging from 10 to 50 µL. The % RSD values for tailing factor and theoretical plates were calculated and the data given in Table 1.

Table 1: Data for calibration curve.

Concentration (µg/mL)	Mean peak area with ± SD
5	51465.67±7.094
10	103390.7±10.598
15	157807±18.027
20	212455.7±99.899
25	261380.3±46.047
REGRESSION EQUATION	y = 10578x –1368.4
R	0.999
R^2	0.9999

Accuracy/recovery

Accuracy is the measure of closeness the experim ental value to it's the true value. It should be established across the specified range of the analytical procedure. To confirm the accuracy of the proposed method recovery studies were performed by spiking 80% (8 µL), 100% (10 µL) and 120% (12 µL) of the standard to the synthetic solution of drug product.

Limit of detection and quantification

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Precision

Precision was determined in terms of repeatability of application (System precision) and measurement (Method precision). The precision of the method was ascertained from the peak areas of six replicate injections of a fixed standard concentration.

Repeatability of standard application was assessed by using six replicates of concentration at 10µg/mL level. The standard deviation and the relative standard deviation were computed for precision.

Robustness

Robustness of the proposed method was determined by analyzing the standard solution at normal operating conditions and by deliberately changing some of the conditions such as flow rate, column, oven temperature, detection wavelength and the mobile phase.

Assay of the formulation

Randomly picked twenty tablets were weighed individually and finely powdered. A powder blend equivalent to 10 mg of TDL was transferred to a 10 mL volumetric flask containing about 5mL of methanol, sonicated and made up with the same solvent. The solution was filtered through 0.45 µm nylon membrane filter to obtain a stock solution of 1mg/mL. It was further diluted with diluent to get the required concentration (10 µg/mL of TDL). The solution was injected three times into the column.

RESULTS AND DISCUSSIONS

Method development and optimization

In order to optimize the LC conditions for the estimation of TDL in bulk, tablets and *in vitro* dissolution samples analysis, the following trials were performed. Initially a mobile phase consisting of Water: Methanol (50:50 %v/v) at a flow rate of 1.0 mL/min was used on an Agilent Eclipse C_{18} column (150 x 4.6 mm, 5µ) column at ambient temperature using methanol as a diluent, TDL did not elute under these conditions. In the next trial, same column was employed but the mobile phase was changed to ammonium acetate and methanol (50:50 v/v), TDL eluted at retention time of 2.84 min but peak parameters were not observed optimal (tailing factor >2). To obtain better peak properties diluents was switched to 10 mM ammonium acetate, TDL eluted with good symmetry and peak properties (tailing factor-1.102) at a retention time of 6.38. In order to further optimize the retention time of TDL, the percentage of organic modifier, methanol was increased and a mobile phase composition of 10 mM Ammonium acetate: Acetonitrile (70:30% v/v), the peak eluted at 0.63 min. Finally 10 mM Ammonium acetate: Methanol (35:65% v/v) was used as mobile phase and a symmetrical peak was observed at the retention time of 4.56 min.

Method validation

Specificity

From the base shift ovelay of the chromatograms in (Figure 3) and 3D plots of diluents, standard, placebo and formulation shown in (Figure 4), it can be inferred that there were no co eluting interfering peaks with the drug peaks (Figure 5).

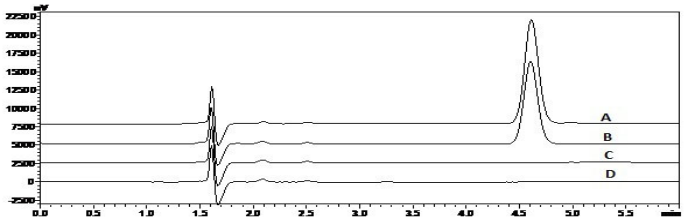

Fig. 3: Overlay Chromatogram of TDL standard (A), sample (B), placebo (C) and diluent (D) chromatogram.

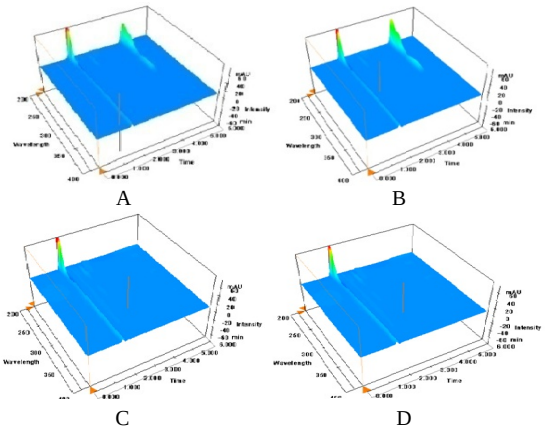

Fig. 4: 3D plot of Standard(A), sample (B), placebo (C) and diluent (D).

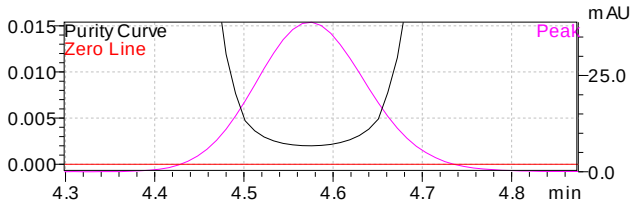

Fig.5: Peak purity Index of TDL (* Peak Purity Index:1.0000 ; Single Point Threshold :0.997084; Min.peak purity index: 2916)

Linearity

The Linearity was established at the concentration range of 5-25 µg/mL for TDL. Peak areas (y) of TDL were plotted versus their respective concentrations (x) and linear regression analysis performed on the resultant calibration curves. Linearity data was shown in Table 1 and Figure 6. Calibration curve depicted in figure 7.

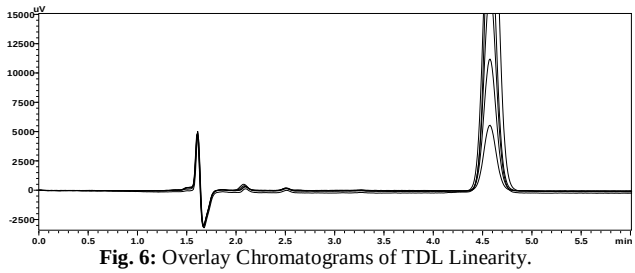

Fig. 6: Overlay Chromatograms of TDL Linearity.

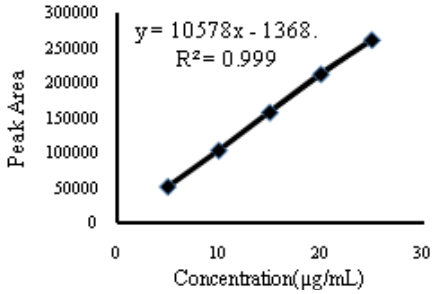

Fig.7: Calibration curve of TDL

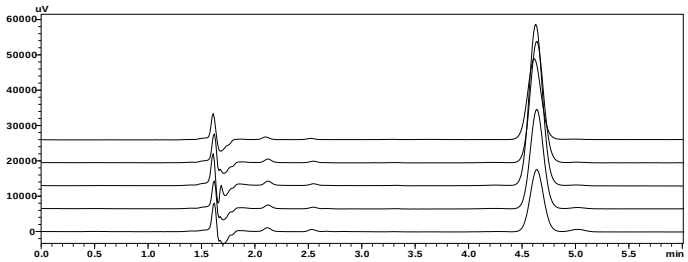

Fig. 8: Overlay of System suitability chromatograms of standard solution (5-25µL) of TDL.

System suitability

The RSD values for system suitability test parameters like retention time [R_t = 4.6262 (1.0207%)], tailing factor [T_f = 1.0826 (0.2224 %)] and theoretical plate number [5259.115 (1.7864 %)] were found to be less than 2 % indicating the present conditions were suitable for the analysis of TDL in tablets. The overlay chromatogram of system suitability is given Figure 8.

Accuracy

The recovery of the standard added to the drug product sample was calculated and it was found to be 99.26-100.97 %. The % RSD was less than 2. The data was given in Table 2.

Table 2: Data for recovery studies.

Level of Recovery	Amount Present	Amount added((µg/mL)	% Recovery (Mean ± SD)	%RSD
80%	10	8	99.26± 1.021	1.028
100%	10	10	100.97±0.599	0.593
120%	10	12	100.090± 1.761	1.76

Limit of detection and quantification

LOD and LOQ for TDL were determined according to ICH guideline Q2 (R1) . LOD and LOQ were found to be 0.009µg/mL and 0.0272µg/mL respectively, indicating good sensitivity of the method.

Precision

System precision and method precision was confirmed as the % RSD were well within the target criterion (<2%). Precision data was shown in Table 3 and Figure 9.

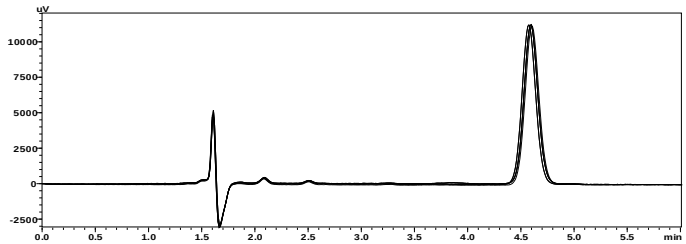

Fig. 9: Overlay Chromatograms of TDL Precision.

Table 3: Precision data of TDL-10µg/mL (n=6).

Validation parameter	Area		Retention Time	
	Mean ± SD	%RSD	Mean ± SD	%RSD
System Precision	103709.7±290.3595	0.279	4.585 ± 0.0135	0.2929
Method Precision	261489.7±429.2494	1.6415	4.596±0.00954	0.2073

Robustness

Robustness of the method was determined by analyzing the standard solution at normal operating conditions and by changing some of the conditions. The conditions varied and their results were shown in Table 4. The tailing factor is below 2 which is acceptable.

Table 4: Table for robustness.

Chromatographic parameter	Retention time (min)	Theoretical plates (#)	Tailing factor (T_f)	%Assay
Mobile phase				
63-37	3.36	10020	1.24	101.4
65-35	3.18	10179	1.25	100.8
67-33	2.87	12412	1.24	100.2
Flow rate				
0.8	3.40	10030	1.26	99.43
1.0	3.16	10179	1.25	100.7
1.2	2.78	12432	1.26	100.23
Wave length (nm)				
279	3.16	10181	1.24	101.2
280	3.16	10179	1.25	99.8
281	3.16	10179	1.25	100.12

Assay of the marketed formulation

Assay of TDL tablets was performed by the proposed method and the % assay of the formulation was calculated as an average of 3 determinations. The data is given in table 5. The assay was found to be within the limits, indicating that the present LC conditions can be used for the assay of TDL in different commercially available formulations.

Table 5: Data for Assay.

Formulation	Labelled Amount (µg)	Amount found (mg) (Mean ± SD)	% Assay	% RSD
megalis10mg	10	10.037±0.073	100.379	0.728

Dissolution studies

The dissolution experiments were conducted in triplicate as per the method described above. The amount of TDL in the test samples was calculated as percentage drug dissolved, by comparing peak area of the test samples with standard. The dissolution parameters and data for 6 tablets were given in table 6 and 7. The dissolution profile was depicted in figure 10.

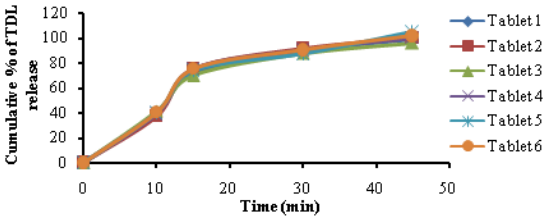

Fig. 10: Dissolution profile of TDL.

Table 6: Dissolution parameters.

S.N	Time (min)	Peak area	Concentration (µg/mL)	Cum drug release	% of drug release	% of drug unreleased
1.	0	-	-	-	-	-
2.	10	45431.6666	4.3941	3.9547	39.5475	60.45
3.	15	84436.8333	8.1667	7.3500	73.5009	26.5
4.	30	102543.5	9.9180	8.9262	89.2625	10.74
5.	45	114891	11.1123	10.0010	100.0108	0

Table 7: Percentage release of TDL.

Time	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 5	Tablet 6
0	0	0	0	0	0	0
10	39.37201315	37.3255	41.408	38.8209	39.9369	40.4218
15	72.17970282	74.7989	69.5403	75.5249	73.6238	75.3378
30	89.65293784	91.3146	87.2695	90.1308	87.0207	90.1369
45	98.79824781	99.9977	95.7454	99.6513	104.4198	101.4523

Filter compatibility study

Compatibility of 0.45µm nylon filter and 0.45µm PVDF filter was studied. Standard sample solution and dissolution samples were filtered and analysed and the variation in the assay value was calculated and tabulated in Table 8. After the analysis it was found that nylon filters are suitable for filtration.

Table 8: Data for Filter compatibility study.

Sample name	Peak area of TDL	% Difference
Standard sample	46442	-
Samples filtered through 0.45µm nylon filter	46436	0.012

CONCLUSION

The simple, rapid, sensitive, and inexpensive isocratic RP-HPLC method was developed and validated for the estimation of TDL in bulk and pharmaceutical formulation and dissolution samples. The dissolution study showed that TDL has good stability and the percentage of drug released was satisfactory for all the evaluated batches from the formulation. The method was in compliance with all validation parameters. Therefore this method is proposed for the quality control of TDL in bulk and its pharmaceutical dosage forms contributing to assuring the therapeutic efficacy of the drug.

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