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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF TADALAFIL IN BULK AND TABLET FORMULATION

Kharat S.S.*, Andhale S.P. and Saudagar R.B.

R.G. Sapkal College of Pharmacy, Anjaneri, Kalyani Hills, Nasik.

***Corresponding Author: Kharat S.S.**
R.G. Sapkal College of Pharmacy, Anjaneri, Kalyani Hills, Nasik.

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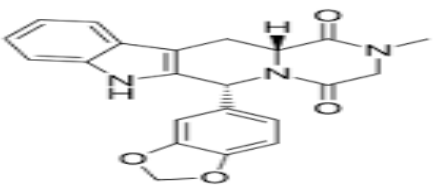
ABSTRACT
The simple, selective, accurate, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for analysis of tadalafil in bulk and tablet dosage form. The separation was carried out on Hexon C18 (250mm x 4.6ID, Particle size: 5 micron) in isocratic mode column using the mobile phase composition was Methanol and Water (80:20% v/v) adjusted pH to 3.0 with orthophosphoric acid. The injection volume was 20µl and detection wavelength was used 220 nm. The flow rate was 0.8 ml/min and effluent was detected at 220 nm with a sharp peak was obtained for TDL at a retention time of 4.8 ± 0.01 min. Linearity was observed in the concentration range from 15 to 75 µg/ml for tadalafil with a correlation coefficient of (r2) 0.9994. The percentage recovery was within the range between 99.62 to 100.43% for tadalafil. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. The method was validated according to International Conference on Harmonisation guidelines in terms of linearity, accuracy, precision and specificity. Hence, the proposed method can be utilized for routine quality control of tadalafil in bulk and tablet dosage form.

KEYWORDS: International conference on harmonisation guidelines, tadalafil (TDL), ultraviolet detection, validation, reverse phase high performance liquid chromatography.

INTRODUCTION
Tadalafil is a cyclic guanosine monophosphate (cGMP), selective inhibitor of specific Type V phosphodiesterase (PDE5) and mainly used for erectile dysfunction and pulmonary arterial hypertension. Chemically, tadalafil is (6R-trans)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino (1',2':1,6.) pyrido (3,4-b) indole 1,4-dione and its empirical formula is $C_{22}H_{19}N_3O_4$. A detailed literature survey suggests various methods for estimation of tadalafil in bulk and blood plasma such as capillary electrophoresis, high performance liquid chromatography electron impact mass spectrometry and ultraviolet (UV) spectroscopy. The present research was undertaken to develop novel, accurate, precise, fast and chief liquid chromatographic method for the estimation of tadalafil in bulk and tablet dosage form.



Structure of tadalafil.

To optimize the reverse phase HPLC (RP-HPLC) parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for tadalafil was obtained with a mobile phase consisting of Methanol and Water (HPLC grade) on 80:20 % v/v which was adjusted to pH 3.0 with orthophosphoric acid. Quantification was achieved with UV detection at 220 nm based on peak area and retention time was found 4.8 min. Suitability of chromatographic system was monitored by calculating tailing/asymmetry factor and theoretical plates.[1,2,4,6]

MATERIALS AND METHOD
Reagents and Materials
Chemicals and reagents: Standard gift sample of Tadalafil was procured from Glenmark Pharma Ltd., Methanol (HPLC grade) and Water (HPLC grade) was obtained from Merck Laboratories Pvt. Ltd., Mumbai.

Instrument
HPLC 3000 Analytical Technologies Ltd. binary gradient pump and UV detector is used. The system was controlled through HPLC Workstation software using Hexon C18 (4.6 x 250 mm, 5µm) column.

Preparation of mobile phase

The mobile phase was composed of methanol: water (80:20 v/v) and adjusted at pH3 using O-Phosphoric acid.

Chromatographic conditions

The data acquisition was performed by HPLC Workstation Software. The analysis was performed by using HPLC column Hexon C18 (250mm x 4.6ID, Particle size: 5 micron) with flow rate of 0.8 ml/min and at ambient temperature. The mobile phase composition was Methanol and Water (80:20% v/v) adjusted pH to 3.0 with orthophosphoric acid. The injection volume was 20µl and detection wavelength was used 220 nm. Mobile phase was filtered through 0.22 µm nylon filter (Millipore) using filtration assembly with vacuum pump P-3000-M Reciprocating (40MPa) and ultrasonicated using ultrasonic water bath (Wenser Ultra Sonicator) for degassing.

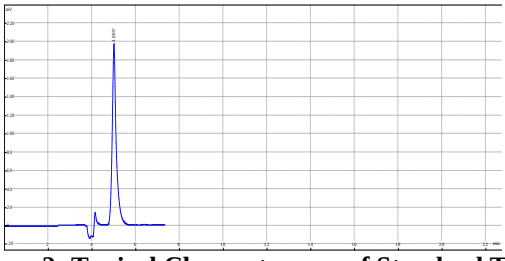


Figure 2: Typical Chromatogram of Standard TDL

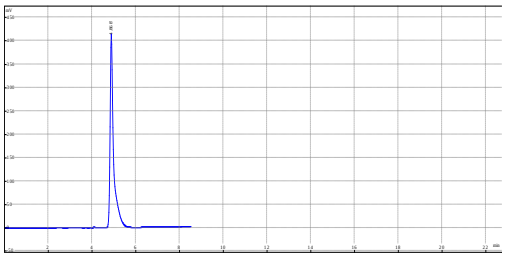
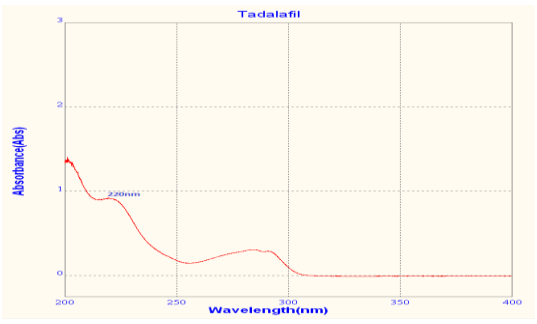


Figure 3: Chromatogram of Tadalafil (TDL) in Tablet Formulation

Determination of wavelength of maximum absorbance

The standard solutions of Tadalafil were scanned in the range of 200 - 400 nm against mobile phase as a blank. Tadalafil showed maximum absorbance at 220 nm. So the wavelength selected for the determination of Tadalafil was 220 nm.



UV Absorbance Spectra of Tadalafil at 220 nm

Standard Stock Solutions

For HPLC analysis 10 mg of Tadalafil powder was weighed accurately using wencer high precision balance (readability 0.01 mg) and transferred in to 10 mL volumetric flask, dissolved and diluted to 10 mL with mobile phase and ultrasonicated for 5 min to produce stock solution containing 1000 µg/ mL of Tadalafil. From stock solution further dilutions prepared 0.15, 0.30, 0.45, 0.60, 0.75 ml stock solution take and make up the volume up to 10 ml gives µg/ mL concentration respectively.

Calibration curve of Tadalafil

Appropriate aliquots of standard stock solutions of TDL were diluted with mobile phase to obtain concentrations in the range of 15, 30, 45, 60 and 75 µg/ml of TDL. The linearity of TDL was found to be in the concentration ranges of 15-75 µg/ml, (Table 1). The coefficient of correlation was found to be 0.9994. An aliquot (20 µl) of each solution were injected under the operating chromatographic condition and chromatogram was recorded.

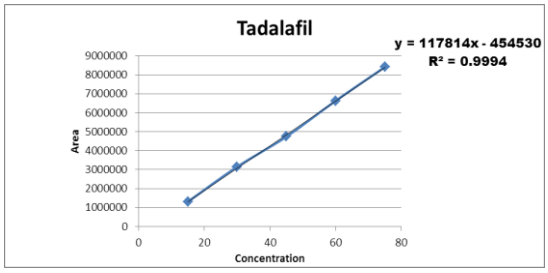


Fig 1: Linearity plot of TDL

Table 1: Linearity data of TDL

Conc. (µg/ml)	Area
15	1313540
30	3147439
45	4738035
60	6626341
75	8410136

System Suitability

In order to determine the adequate resolution and reproducibility of the proposed methodology, suitability parameters including retention time, asymmetry factor, %RSD of retention time and peak areas were investigated. The results are summarized in Table 2.

Table 2: System Suitability

Parameter	Tadalafil (TDL)
Linearity Range (ug/ml)	15-75ug/ml
Correlation of coefficient (r^2)	0.9994
Limit of Detection (ug/ml)	3.36 µg/ml
Limit of Quantitation (ug/ml)	10.01µg/ml
Retention Time (min)	4.9 min
Tailing Factor	1.39
Therotical plates	5432

Analysis of tablet formulation

Twenty tablets of TDL (Tadacip 20 mg) were weighed and their mean weight was determined. The tablets were grinded to fine powder in glass mortar. The powder sample equivalent to 10 mg of TDL was weighed and transferred into a 100 ml volumetric flask and dissolved in 50 ml methanol (HPLC grade). The flask was kept in an ultrasonic bath for 20 min. The volume was adjusted to 100 ml with methanol (HPLC grade). The solution was filtered through 0.2 µ nylon membrane filter. From this stock solution, 4.5 ml solution was pipetted out and transferred to 45 ml and made volume up to the mark with mobile phase to get the concentration 45 µg/ml of TDL. The solution was injected into HPLC system (Fig.3).

The results of the assay of tablet formulation and its statistical validation data are given in Table 3.

Table 3: Assay analysis

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	**% Assay**	4738035	4684959	98.8798

The proposed method was validated according to the International Conference on Harmonisation (ICH) guidelines with respect to linearity range, accuracy, precision (repeatability and intermediate), sensitivity and specificity.

Accuracy

The accuracy of the method was determined by calculating recovery of tadalafil by the standard addition method. The accuracy of the method was determined by preparing solutions of different concentrations 80, 100 and 120% level to prequantified sample solutions of tadalafil (15, 45 and 75 µg/ml, respectively). The solutions were prepared in triplicates and the accuracy was indicated by percentage recovery.

Table 4: Accuracy Study for TDL

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc. (µg/ml)**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	15	1313540	1320171	13452.7696	1.0190172	1.01901720
	15	1311321				
	15	1335652				
2	45	4738035	4740785.66	50254.4906	1.0600456	1.06004561
	45	4792359				
	45	4691963				
3	75	8410136	8387815	19398.3582	0.2312683	0.23126831
	75	8378275				
	75	8375034				

Recovery Study

The Percent Recovery was found within limit.

Table 5: % Recovery Study for Tadalafil

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Recovery
1	**50% Recovery**	4738035	4758531	100.4325
2	**100% Recovery**	6626341	6601263	99.6215
3	**150% Recovery**	8410136	8419978	100.1170

Precision

The precision of the method was checked by inter day and intraday repeatability and reproducibility. The repeatability of method was analysed by repeatedly injecting (n=6) solutions of tadalafil (45 µg/ml) into the HPLC system. The results are shown in the table 6 and 7, which indicates that the proposed method is good with high precision. Moreover, the low RSD values indicate the high degree of correctness of method.

Table 6: Interday and Intraday Precision of TDL Interday

Day 1			Day 2			Mean	%RSD
4738035	4792359	4691963	4778516	4778392	4757478	4757478	**0.77%**

Intraday

Morning			Evening			Mean	%RSD
4738035	4792359	4691963	4760387	4721474	4721497	4737619	**0.74%**

Robustness

Robustness is the ability of method to remain unaffected by small changes in parameters. Here in this study different mobile phase compositions and wavelength variations (± 1 nm) were investigated.

The results revealed that the method is robust enough.

Table 7: Robustness study for method

Sr. No.	Conc.	Area	Mean	SD	%SD
1	60	6660862	6644661	24182.8	0.36394289
	60	6616864			
	60	6656257			

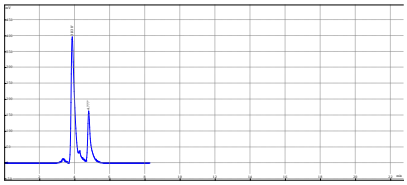
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentration of standard solution by using the HPLC method. The LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD=3.3×σ/S and LOQ=10×σ/S, where σ is slope and S is standard deviation.

Forced degradation Studies

Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analysed for peak purity of Tadalafil peak. In all the samples, Peak purity meets the acceptance limits.

Table 8: Degradation with Peroxide

Concentration	45 ppm
Treated	3%H2O2 at RT for 24Hrs
Wavelength	220nm
Mobile Phase	Methanol: Water (80:20)
pH	3.0
Sample volumes	20µl
Flow rate	0.8 ml/min
Pressure	9-10 MPa
Run time	8.24min

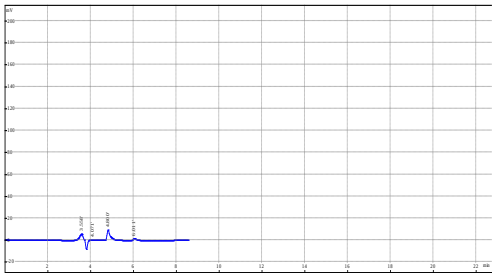


Symmetry

Figure 4: Degradation of TDL with Peroxide

Table 9: Degradation with Acid

Concentration	45 ppm
Treated	0.1N HCl at 60°C for 15min
Wavelength	220nm
Mobile Phase	Methanol: Water (80:20)
pH	3.0
Sample volumes	20µl
Flow rate	0.8 ml/min
Pressure	9-10 MPa
Run time	8.57min



Asymmetry

Figure 5: Degradation of TDL with Acid

Table 10: Degradation with Alkali

Concentration	45 ppm
Treated	0.1N HCl at 60°C for 15min
Wavelength	220nm
Mobile Phase	Methanol: Water (80:20)
pH	3.0
Sample volumes	20µl
Flow rate	0.8 ml/min
Pressure	9-10 MPa
Run time	11.85min

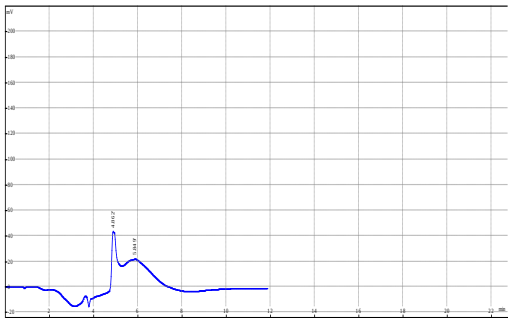
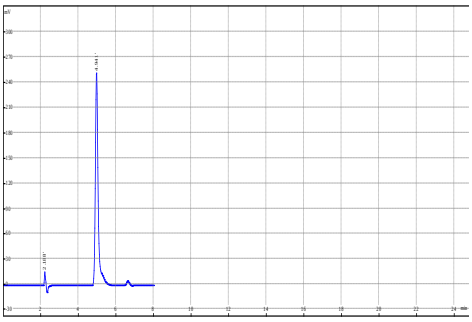


Figure 6: Degradation of TDL with Alkali

Table 11: Photolytic Degradation

Concentration	45 ppm
Treated	Photolytically at RT for 24Hrs
Wavelength	220nm
Mobile Phase	Methanol: Water (80:20)

pH	3.0
Sample volumes	20µl
Flow rate	0.8 ml/min
Pressure	9-10 MPa
Run time	8.00min

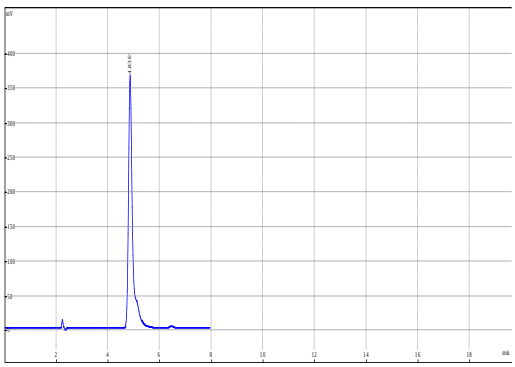


Asymmetry

Figure 7: Degradation of TDL with Photolytic

Table 12: Thermal Degradation

Concentration	**45 ppm**
Treated	Thermally at 60^{o}C for 24Hrs
Wavelength	220nm
Mobile Phase	Methanol: Water (80:20)
pH	3.0
Sample volumes	20µl
Flow rate	0.8 ml/min
Pressure	9-10 MPa
Run time	7.92min



Symmetry

Figure 8: Degradation of TDL Thermally

CONCLUSION

A simple, precise, selective, sensitive and rapid RP-HPLC method with UV detection for tadalafil in pharmaceutical dosage form has been developed and validated. The method has been found best than from few methods reported, because of use of a less economical and readily available mobile phase, lack of extraction procedures. The method would be extensively used for the estimation of tadalafil in bulk and pharmaceutical formulation. Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analysed for peak purity of Tadalafil peak. In all the samples, Peak purity meets the acceptance limits.

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