Rapid Estimation of Tadalafil by Reverse-phase High-performance Liquid Chromatography Method in Bulk and Tablet Formulation

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The simple, selective, precise and accurate reverse-phase high-performance liquid chromatography method was developed and validated for analysis of tadalafil in bulk and tablet dosage form. The column was Inertsil C18 (150×4.6 mm; 5 μm) in isocratic mode. The mobile phase used was phosphate buffer (10 mM, pH 3.2) and acetonitrile (50:50% v/v) at the flow rate of 1.0 ml/min with ultraviolet detection at 295 nm at ambient temperature. The retention time for tadalafil was found to be 4.01 min. Linearity was observed in the concentration range from 60 to 140 μg/ml for tadalafil with a correlation coefficient of (r^2) 0.9998. 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.

Key words: International conference on harmonisation guidelines, reverse phase high performance liquid chromatography, tadalafil, ultraviolet detection, validation

Tadalafil is mainly used for erectile dysfunction and pulmonary arterial hypertension. It works by inhibiting the enzyme phosphodiesterase type 5 and cyclic guanosine monophosphate. 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 $C_{22}H_{19}N_3O_4$ (fig. 1). Literature survey suggests several methods for estimation of tadalafil in bulk and blood plasma such as capillary electrophoresis, high performance liquid chromatography (HPLC) electron impact mass spectrometry and ultraviolet (UV) spectroscopy[1-4]. The present investigation was undertaken to develop new, accurate, precise and fast liquid chromatographic method for the estimation of tadalafil in bulk and tablet dosage form.

Tadalafil was supplied by Srini Pharmaceuticals Ltd., Choutuppal, India. Acetonitrile, potassium dihydrogen-orthophosphate (AR) and orthophosphoric acid were procured from Merck Ltd., Mumbai. High purity water was prepared using Milli Q grade purification system.

The HPLC system of Shimadzu (LC-2010CHT) with UV detector was used for analysis. The data acquisition was performed by LC Solution Software. The analysis was performed by using HPLC column Inertsil (150×4.6 mm; 5 μm) with flow rate of 1 ml/min and at ambient temperature. The mobile phase composition was phosphate buffer (10 mM) adjusted pH to 3.2 with orthophosphoric acid and acetonitrile (50:50 v/v). The injection volume was 20 μl and detection wavelength was used 295 nm. Mobile phase was filtered through 0.22 μm nylon filter (Millipore) using filtration assembly with vacuum pump (Rocker pump 400, Today's) and ultrasonicated using ultrasonic waterbath (Model UCB 100, Spectralab) for degassing.
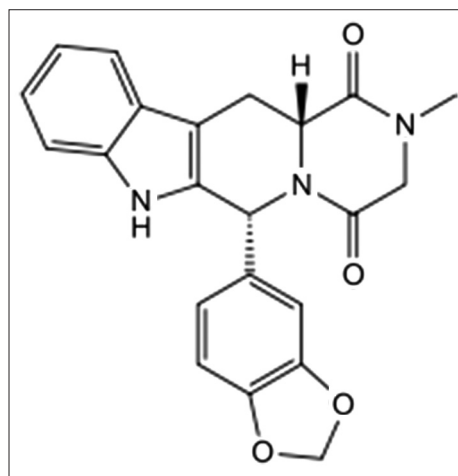

Fig. 1: Structure of tadalafil.

Accurately weighed quantity of tadalafil (25 mg) was transferred to 100 ml volumetric flask containing mobile phase, ultrasonicated for 5 min and volume was made with mobile phase to give 250 μg/ml. The stock solution was further diluted sufficiently to give 50 μg/ml solution.

Twenty tablets of tadalafil (Zydalis, 20 mg) were weighed and their mean weight was determined. The tablets were grinded to fine powder in glass mortar. Accurately weighed powder equivalent to 25 mg of tadalafil was transferred in 100 ml volumetric flask containing mobile phase, solution was sonicated for 15 min and volume was made up to mark with mobile phase. The solution was then filtered through a 0.45 μm filter and sufficiently diluted to give concentration of 50 μg/ml.

The proposed method was validated according to the International Conference on Harmonisation (ICH) guidelines[5,6] with respect to linearity range, accuracy, precision (repeatability and intermediate), sensitivity and specificity.

Calibration curve was constructed by plotting peak area versus concentration of tadalafil solutions and the regression equation was calculated. The calibration curve was plotted over the concentration range of 30-70 µg/ml. Aliquots of 3, 4, 5, 6 and 7 ml of 250 µg/ml stock solutions were transferred to series of 25 ml volumetric flask and volume was made up to mark with mobile phase to give different concentrations, ranging from 30 to 70 µg/ml. An aliquot (20 µl) of each solution were injected under the operating chromatographic condition and chromatogram was recorded. A representative chromatogram is shown in fig. 2.

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 that is 80, 100 and 120% level to prequantified sample solutions of tadalafil (40, 50 and 60 µg/ml, respectively). The solutions were prepared in triplicates and the accuracy was indicated by percentage recovery.

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of tadalafil (50 µg/ml). The intermediate precision of the method was determined on different day by using column of different make of same dimensions. The standard solution (50 µg/ml) was injected for 6 times and area was measured. The percentage relative standard deviation (%RSD) for all injections was within the specified limits.

Robustness, a deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The results revealed that the method is robust enough. The limit of detection (LOD) and limit of quantificatio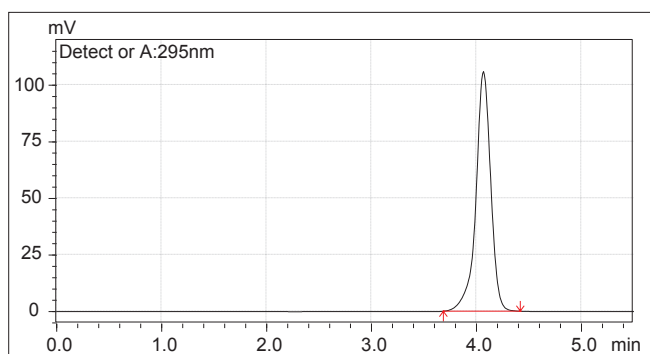n (LOQ) of the developed method were determined by injecting progressively low concentration of standard solution by using the HPLC method. The LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD=3.3×σ/S and LOQ=10×σ/S, where σ is slope and S is standard deviation.

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 phosphate buffer (10 mM, pH 3.2): Acetonitrile (50:50 v/v). Quantification was achieved with UV detection at 295 nm based on peak area and retention time was found 4.01 min. Suitability of chromatographic system was monitored by calculating tailing/asymmetry factor and theoretical plates.

The calibration graph was linear, the system adhered to Beer's law over the range 30-70 µg/ml (r^2=0.9998). Linearity was evaluated by triplicate analysis of five standard working solutions ranging from 30 to 70 µg/ml. The overlain chromatogram of tadalafil at 295 nm is shown in LOD and LOQ were estimated from the residual standard deviation. The LOD and LOQ for tadalafil was 1.19 and 3.61 µg/ml, respectively. This indicates that the method is sensitive for the determination of tadalafil. System suitability and validation parameters are given in Table 1. Accuracy of method was calculated using recovery studies and percentage recovery was calculated, which is in good agreement with the labelled claims. High percentage recovery showed that the method is free from interference of the excipients used in formulations and is accurate. The results are given in Table 2. The low %RSD values of system and method precision for tadalafil

Fig. 2: Typical chromatogram of tadalafil at 295 nm.

TABLE 1: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Linearity range (µg/ml)	30-70
Slope (m)	4.81518
Intercept (c)	−0.27857
Correlation coefficient (r^2)	0.99986
Retention time (min)	4.01
Area	1039090
Height	105849
Tailing factor	0.9823
Theoretical plates	4097.379
LOD (µg/ml)	1.19
LOQ (µg/ml)	3.61

Area, height, tailing factor and theoretical plates (50 µg/ml). LOD=limit of detection, LOQ=limit of quantification

TABLE 2: RECOVERY STUDY OF TADALAFIL (TABLET) *N*=3

% concentration (at specification level)	Area	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	Mean recovery
80	838587.5	40	40.376±0.07	100.94±0.13	
100	1041101	50	50.125±0.12	100.25±0.03	100.65
120	1255529	60	60.450±0.04	100.75±0.08	

TABLE 3: INTERMEDIATE PRECISION AND SYSTEM SUITABILITY STUDIES OF TADALAFIL

Precision	Day 1	Day 2
System precision (%RSD)	0.033	0.038
Method precision (%RSD)	0.051	0.071
Flow rate (ml/min)	System suitability results RSD (%)	
0.9	0.0351	
1	0.0332	
1.1	0.0334	
Change in organic composition in the mobile phase		
10% less	0.0343	
*Actual	0.033	
10% more	0.0337	

*Results for actual mobile phase composition (50:50 acetonitrile:phosphate buffer) have been considered. RSD=relative standard deviation

revealed that the proposed method is precise and intermediate precision is shown in Table 3. Influence of small changes in chromatographic conditions such as change in flow rate (±10%) and organic content in mobile phase (±10%) studied to determine the robustness of the method and are also in favour of the developed method. The results are given in Table 3. Specificity was performed to exclude the possibility of interference with excipients in the region of elution of tadalafil. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of tadalafil. The validated HPLC method was adopted for the quantification of tadalafil tablet dosage form were found to be in the range of 100±5% with RSD less than 2%, which indicate

suitability of the method for routine analysis of tadalafil in tablet dosage form.

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.

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