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Development and validation of a rapid RP-HPLC method for the determination of tadalafil in bulk and in formulation

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for assay of Tadalafil in bulk and in tablet dosage forms. Isocratic elution at a flow rate of 0.9 ml/ min was employed on a Phenomenax Luna C_{18} column (150×4.6 mm; 5µ) at ambient temperature. The mobile phase consisted of acetonitrile and water (50:50% v/v). The detection wavelength was 295nm and 25µl of sample was injected. Lamotrigine was used as an internal standard (IS). The retention times for TDF and IS were 4.12 and 2.29 min, respectively. The method obeys Beer's law in the concentration range of 1-5µg/ml. The method was successfully applied to commercial pharmaceuticals and validated as per standard analytical procedures. The proposed method could be applicable for routine analysis of TDF in bulk and in formulations.

KEYWORDS

Tadalafil;
Lamotrigine;
RP-HPLC;
UV detection;
Internal standard.

INTRODUCTION

Tadalafil (TDF)[1-3] is a selective, reversible inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5). It is the drug mainly used for erectile dysfunction. The structure of TDF shows it possesses a hydrophobic moiety. When sexual stimulation causes the local release of nitric oxide, inhibition of PDES by TDF produces increased levels of cGMP in the corpous cavernosum. This results in smooth muscle relaxation and inflow of blood into the penile tissues, there by producing an erection.

Hitherto there are few analytical methods reported for estimation of TDF. The determination of TDF in small volumes of plasma by HPLC with UV detection was described[4]. Quantitation of TDF in human plasma was reported by HPLC-tandem mass spectrometry with electrospray ionisation[5]. Sildenafil, verdenafil and TDF were determined simultaneously by HPLC-EIMS in human plasma and urine[6]. These methods are complicated, costly, time consuming rather than a simple HPLC with UV detection. So it is unsuitable to use these highly sensitive methods for the routine quantitative assay of TDF in bulk and in tablets where the content of active pharmaceutical ingredient is high in the formulation.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of TDF

in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time(less than 5 min) with IS eluted prior to TDF, short analysis time(less than 30 min); good precision (R.S.D. less than 2%) and high recovery(greater than 95%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH), to determination of TDF in bulk and in tablet dosage form has been also performed.

EXPERIMENTAL

Chemicals and reagents

HPLC grade acetonitrile and water was purchased from Qualigens fine chemicals (Mumbai, India). Tadalafil standard sample (Figure 1) was provided by Aurobindo Pharma Limited (Hyderabad, India). The internal standard, Lamotrigene (Figure 1) was obtained from Jubilant Pharma Limited (Mumbai, India). Forzest and Tazzle commercial formulations selected and they belong to Ranbaxy Laboratories Limited (New Delhi, India) and Dr.Reddy's Laboratories (Hyderabad, India), respectively. All of the commercial samples were supplied as tablet dosage forms containing 20mg of TDF for oral administration. The molecular weight is 389.40 for TDF.

Instrumentation and analytical conditions

The HPLC system (Shimadzu, Japan) consisted of a pump (LC-10 ATVP series pump) equipped with a Rheodyne model 7161 injection valve with a 20µl loop (Rheodyne Inc., Cotati, CA, USA), an UV-visible detector (SPD 10 AVP) set at 295nm. The analytical column, a Phenomenax Luna C_{18} (150mm×4.6mm i.d., 5µ particle size) was operated at ambient temperature (20±1°C). Isocratic elution with acetonitrile: water (50:50% v/v) was used at a flow rate of 0.9ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use (Soltec, Soluzioni tecnologiche, Luglio, Italy). The UV spectrum of TDF for selecting the working wavelength of detection was taken using a Shimadzu UV-1700, UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan).

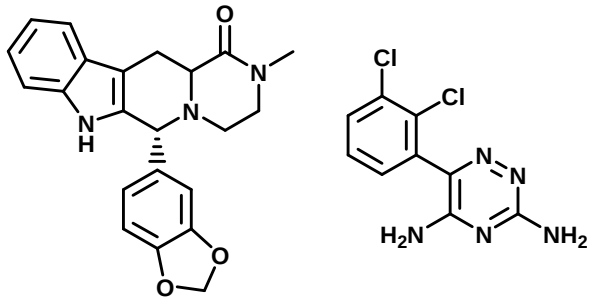

Figure 1 : Chemical structures of (a): Tadalafil and (b): Lamotrigine

Stock and working standard solutions

Standard stock solution of 500µg/ml of TDF was prepared freshly by accurately weighing 25mg of TDF into 50ml volumetric flask. Dissolved and made up to the volume with mobile phase. The solution was diluted by pipetting 2.0ml into 100ml volumetric flask to obtain 10µg/ml solution. The solution was further diluted with mobile phase in 10ml volumetric flask to obtain five working standards in the concentration range of 1,2,3,4 and 5µg/ml of TDF covering 33.33-166.65% of the intended test concentration of 3µg/ml for the pharmaceutical formulation. The calibration standards were added with 3.0 ml of freshly prepared 10µg/ml solution of lamotrigene as an internal standard (final concentration, 3µg/ml) and made up to volume with mobile phase. All the solutions were prepared in triplicates. Before being subjected to analysis, all the working standard solutions were filtered through 13 mm membrane syringe filter (Pore size 0.2µm).

Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The calibration curve was plotted with the five concentrations of the 1-5µg/ml working standard solutions. So chromatography was repeated thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay sample preparation

The contents of twenty commercial tablets (labeled concentration 20mg of TDF) were each weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25mg of TDF was quantitatively transferred into a 50

ml volumetric flask with about 45ml of mobile phase. The solution was sonicated for 10 min, brought to the volume with mobile phase, mixed well and filtered through 13 mm membrane syringe filter (pore size 0.2μm). A 2.0ml filtered test solution was transferred into 100 ml volumetric flask and made up to the volume with mobile phase (10μg/ ml). A 3.0ml aliquot was transferred into a 10ml volumetric flask, 3.0ml of a 10μg/ ml solution of IS was added and diluted to volume using mobile phase. The theoretical TDF concentration after dilution was 3μg/ml (100% of TDF). An aliquot of this solution was filtered through a 13 mm membrane syringe filter (pore size 0.2μm) prior to the injection into the HPLC system. Peak area ratios of TDF to that of IS were then measured for the determinations.

Validation procedure

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines[7]. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, short term stability and system suitability.

Standard plots were constructed with five concentrations in the range of 1-5μg/ml prepared in triplicates to test linearity. The ratio of peak area signal of TDF to that of IS was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method.

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared TDF test solution in the same equipment at a concentration of 100% (3μg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area ratios of TDF to that of IS were determined and precision was reported as %R.S.D.

Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples of TDF at three different levels (10, 30 and 50%) in pure solutions using three preparations for each level. The results were expressed as the percentage of TDF recovered in the samples.

Specificity was assayed by comparing the chromatograms obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets and verifying the absence of interferences. Sample solution short term stability was tested at ambient temperature (20±1°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

A system suitability test was performed by six replicate injections of the standard solution at a concentration of 3μg/ ml verifying IS/ TDF resolution >2; % R.S.D. of peak area ratios of TDF to that of IS ± 2; % R.S.D. of each peak retention time ± 2%.

RESULTS AND DISCUSSION

Screening and optimization

Selection of the detection wavelength

The overlain UV spectra of TDF and IS in 50:50 % v/v mixture of ACN and water, in the region between 200 and 400nm, are shown in figure 1. It shows that at 295nm, both TDF and IS have marked absorbance. Hence this was selected as an optimum detection wavelength for the quantification of TDF.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug TDF is non polar. Non polar compounds preferably analyzed by reverse phase columns. Among C_8 and C_{18}, C_{18} column was selected. Non polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of Acetonitrile and water was selected as mobile phase and the effect of composition of mobile phase on the retention time of TDF was thoroughly investigated. The concentration of acetonitrile (40-60% v/v) and water (40-50% v/v) were optimized to give symmetric peak with short run time. A short run time and the stability of

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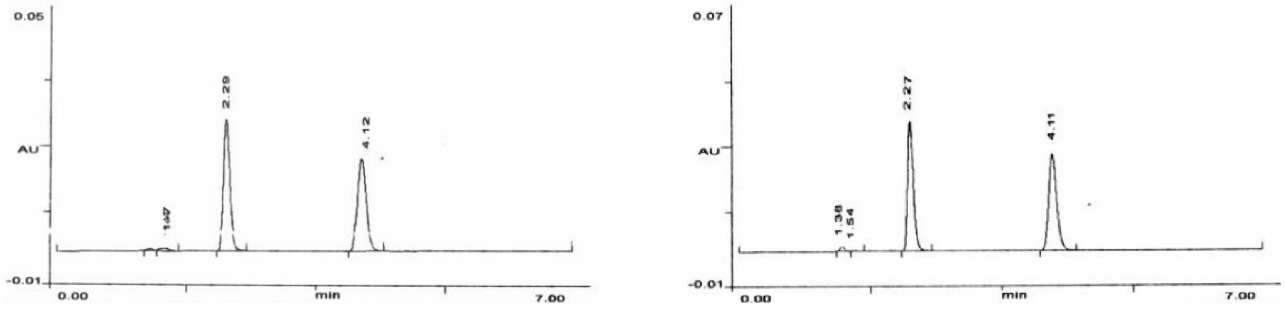

(a) (b)

Figure 2 : Typical chromatograms obtained from the analysis of (a): TDF standard solution (3µg/ml) and (b): TDF extracted from tablets (3µg/ml)containing 3µg/ml of IS. Retention times of IS and TDF were 2.29 and 4.12 min, respectively. The chromatographic conditions were as described in section 2

TABLE 1 : Statistical analysis of calibration curves in the HPLC determination of tadalafil (n = 6)

Validation parameters	Values
Concentration range (µg/ ml)	1-5
Number of concentration range	5
Regression equation	Y=0.3254x+0.3340
Slope(b)	0.3254
Standard deviation on slope (S_b)	0.00896
Intercept (a)	0.3340
Standard deviation on intercept	0.0213
Determination coefficient	0.9999
Residual sum of square	0.0001050
F-value	30186.50

peak asymmetry were observed in the ratio of 50:50% v/v of acetonitrile and methanol. It was found to be the optimum mobile phase concentration.

Choice of internal standard

Several substances were tested as internal standards. Among these, lamotrigine has been chosen as the most appropriate in the present analysis because it is stable. In the present study, it did not interfere with the matrix of pharmaceutical samples and it was well separated from TDF. More over, a significant advantage of this IS was its elution time that was shorter than that of TDF resulting in short run time, less than 5 min. A typical chromatogram of TDF and IS using the proposed method is shown in figure 2. Sharp and symmetrical peak was obtained with good baseline for each compound, thus facilitating the accurate measurements of peak area. The average retention times for TDF and IS were found to be 4.11 ± 0.03 and 2.27 ± 0.02 min, respectively. Under the described parameters, the respective compounds were clearly separated and their

TABLE 2 : Accuracy study for tadalafil

Concentration range (µg/ ml)	Mean recovery		% RSD	
	Forzest	Tazzle	Forzest	Tazzle
0.3	100.34	98.70	0.9616	0.9146
0.6	99.18	100.85	0.9036	1.0349
1.2	100.84	99.74	1.0660	0.9673

corresponding peaks were sharply developed at reasonable retention times.

Validation of methods

Linearity

Five points calibration graphs were constructed covering a concentration range 1-5µg/ml (see section 2.3). Three independent determinations were performed at each concentration. Linear relationships between the ratio of peak area signal of TDF to that of IS versus the corresponding drug concentration were observed, as shown by the results presented in TABLE 1. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.999. To determine whether the experimental intercept (a) of the regression equation was not significantly different from the theoretical zero value, confidence interval (99%) and student's t-test were performed. It concerns the comparison of $t = a/s_a$ where a is the intercept of the regression equation and s_a is the standard deviation of a, with tabulated data of the t-distribution. As the calculated t value (t = 1.4350) does not exceed to (0.001, 14) = 4.140, the intercept of regression equation is not significantly different from 0 (point estimation). By using 99% confidence interval, the value lies between 0.0273-0.0452.This shows that the intercept will fall on this

TABLE 3 : System suitability study

Parameters	Values*	
	TDF	IS
Retention time	4.12	2.29
Area ratio TDF/IS	0.979	0.979
Capacity factor	1.90	0.61
Asymmetrical factor	1.27	1.52
Tailing factor	1.18	1.38
Number of theoretical plates	7780	4879
HETP	0.019	0.031
Resolution	Between TDF and IS 4.55	

***Mean of 10 observations**

range and the distance from zero is very short (Interval estimation).

Precision

The repeatability study (n=6) carried out showed a R.S.D. of 1.340% for the peak area ratio of TDF of IS obtained, thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision a study carried out by the same analyst working on 3 consecutive days (n = 3) indicated a R.S.D. of 0.744 and 1.126%. Both values were far below 2%, the limit percentage set for the precision and indicated a good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of TDF in the real samples. These results are summarized in TABLE 2. The mean recovery data of TDF in real sample were within the range of 99.18 and 100.84% for Forzest and 98.70 and 100.85 for Tazzle. Mean% R.S.D. was 1.348% and 0.880%, satisfying the acceptance criteria for the study.

Specificity

The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak within a retention time range of 5 min. The results showed that the developed method was specific as none of the excipients interfered with the analytes of interest (Figure 2).

Stability

The stability of TDF in standard and sample solutions containing IS determined by storing the solutions

TABLE 4 : Results obtained for determination of tadalafil in tazzle and forzest

Sample	TDF (Theoretical value)* (mg/tab)	TDF (Determined value)* (mg/tab)	Recovery*%	R.S.D.%
Forzest	20	19.85	99.27	1.253
Tazzle	20	19.84	99.21	1.340

***Average of six determinations**

at ambient temperature (20±1°C) protected from light. The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 97%. This denotes that TDF is stable in standard and sample solutions for at least 2 days at ambient temperature, protected from light and is compatible with IS.

System suitability

The resolution factor between IS and TDF, in the developed method, was above 2. The % R.S.D. of peak area ratios of TDF to that of IS and retention times for both drug and IS were within 2% indicating the suitability of the system (TABLE 3). These results indicate the applicability of this method to routine with no problems, its suitability being proved. The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and number of theoretical plates also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of TDF in tablet formulation.

Assay of tablets

The validated method was applied for the assay of two commercial tablets containing 20mg of TDF: Tazzle and forzest. Each sample was analyzed in triplicate after extracting the drug as mentioned in assay sample preparation of the experimental section (section 2.4) and injections were carried out in triplicate Figure2. shows a HPLC chromatogram of TDF in pharmaceutical tablets. None of the tablet ingredients interfered with the analyte peak. The results presented in TABLE 4 are in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, were

found to be 99.21±1.340 for Forzest; 99.27±1.253 for Tazzle showing that the content of TDF in tablet formulations confirmed to the content requirements (95-105%) of the label claim. Low values of standard deviation denoted very good reproducibility of the measurement. The above results demonstrated that the developed method achieved rapid and accurate determination of TDF and could be used for the determination of TDF drug substance and pharmaceutical formulations.

CONCLUSION

A validated isocratic HPLC-UV method has been developed for the determination of TDF in dosage forms Forzest and Tazzle. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic run time of 5 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of TDF in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS that is complicated, costly and time consuming rather than a simple HPLC-UV method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

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