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Full Length Research Paper

Validation and stability indicating RP-HPLC method for the determination of tadalafil API in pharmaceutical formulations

B. Prasanna Reddy[1]*, K. Amarnadh Reddy[2] and M. S. Reddy[3]

[1]Department of Quality control, Nosch Labs Pvt Ltd, Hyderabad-500072, A.P, India.
[2] Department of AR and D, Aurigene Discovery Technologies Ltd, Bangalore, India.
[3]Department of Plant Pathology and Entomology, Auburn University, USA.

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The present study describes the development and subsequent of a stability indicating RP-HPLC method for the analysis of tadalafil. The samples separated on an Inertsil C_{18}, (5 µ , 150 mm x 4.6 mm i.d) by isocratic run using acetonitrile and phosphate buffer as mobile phase), with a flow rate of 0.8 ml/min, and the determination wavelength was 260 nm for analysis of tadalafil. The described method was linear within range of 70 - 130 µg/ml (r^2 = 0.999). The precision, ruggedness and robustness values were also within the prescribed limits (< 1% for system precision and < 2% for other parameters). Tadalafil was exposed to acidic, basic, oxidative and thermal stress conditions and the stressed samples were analyzed by the proposed method. Chromatographic peak purity results indicated the absence of co-eluting peaks with the main peak of tadalafil, which demonstrated the specificity of assay method for estimation of tadalafil in presence of degradation products. The proposed method can be used for routine analysis of tadalafil in quality control laboratories.

Key words: RP-HPLC, tadalafil, validation, stability indicating assay, forced degradation.

INTRODUCTION

Tadalafil hydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino-[1',2':1,6]pyrido[3,4-b]indole-1,4-dione (Figure 1), is a phosphodiesterase type 5 inhibitor used in the management of erectile dysfunction. It is not officially included in any of the pharmacopoeias. It is listed in the Merck Index (Budavari et al., 2001) and Martindle and complete drug reference (Sean et al., 2002). There are several (Cheng et al., 2005) methods for determination of tadalafil such as HPLC-EIMS (Zhu et al., 2005) and capillary electrophoresis methods (Aboul-Enein, 2005) and by HPLC (Aboul, 1994). The present work was designed to develop a simple, precise and rapid analytical LC procedure, which would serve as stability indicating assay method for analysis of tadalafil active pharmaceutical ingredient.

*Corresponding author. E-mail: drbpkreddy@gmail.com. Tel: +91-9848392677.

EXPERIMENTAL

Chemical and reagents

Tadalafil standard and API were provided from Smilax laboratories limited. HPLC grade sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), acetonitrile, hydrogen peroxide and sodium hydroxide were procured from Merck Ltd. High pure water was prepared by using Millipore Milli Q plus purification system.

HPLC instrumentation and conditions

A high performance liquid chromatograph system, with LC solutions data handling system, with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The samples separation was performed on a Shimadzu Inertsil C_{18}, (5 µ, 150 mm x 4.6 mm, Japan) with the mobile phase consisting of acetonitrile and phosphate buffer (pH 7.0) with a ratio of 60: 40 (v/v) at ambient temperature. The flow rate was kept at 0.8 ml/min and the determination wavelength was 262 nm.

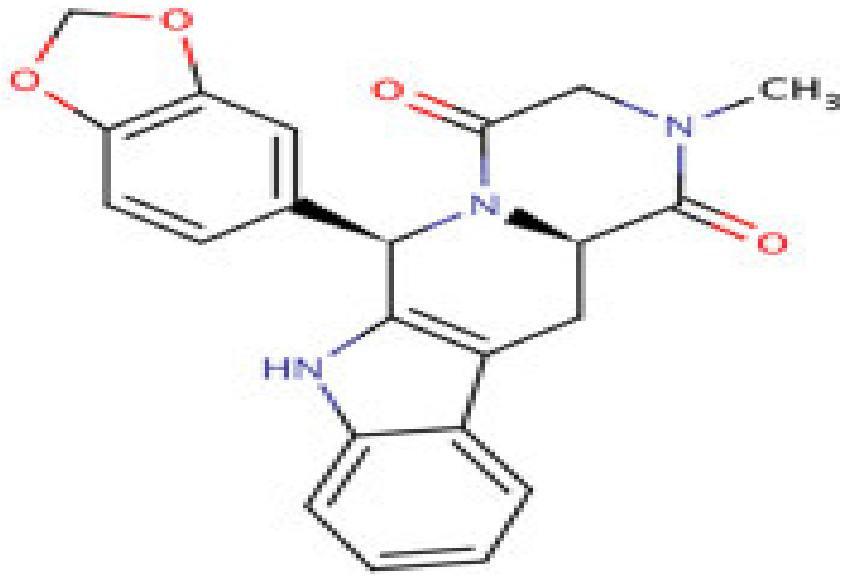

Figure 1. Structure of Tadalafil.

Standard and sample preparation

Mobile phase

Mix 700 ml of acetonitrile to the buffer, the mobile phase was sonicated for 15 min and then it was filtered through 0.45 µm membrane filter paper.

Standard solution

The standard was dissolved with mobile phase to 0.5 mg/ml. The test samples were dissolved with mobile phase. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected and the chromatogram was recorded. This procedure was repeated for the sample solution.

Forced degradation studies

Tadalafil was allowed to hydrolyze in different strengths of base (0.005 N and 0.05 N NaOH), acid (0.05 N, 0.5 N and 1 N HCl) and hydrogen peroxide (30%, 10, 3 and 1%). Tadalafil was also studied for its thermal degradated at 80, 100, 120℃ for 1 h respectively. An accurately weighed 50 mg of tadalafil API was dissolved in 1 ml of respective base (NaOH), acid (HCl) or hydrogen peroxide and kept for specified period after which the volume was made up to 50 ml with water: acetonitrile (70:30, v/v). Five milliliters of the above solution were diluted with water: acetonitrile (70:30, v/v) to get a concentration of 100 ppm. Blank was also treated in same way.

Validation

Linearity was determined by injecting different concentration of sample solutions (50 - 150 µg/ml). For system precision, standard solution (100 µg) was injected to six replicates injections to check %RSD (relative standard deviation) and for method precision six time samples were prepared and each of those were injected in duplicate. Mean of all of these values gives to assay value.

To establish the within-day (intra-assay) and between-day (inter-assay) accuracy and precision of the method, tadalafil was assayed on one day and three separate days. Intra-assay and inter-assay were calculated.

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate (± 10%), organic content in mobile phase (± 2%), wavelength of detection (± 5%) and pH of buffer in mobile phase (± 0.2%). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

Limit of detection (LOD)

The detection limit is determined by the analysis of samples with the known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The LOD was calculated as follows:

$$LOD = 3.3 (\sigma/S)$$

Limit of quantitation (LOQ)

The quantitation limit is determined by the analysis of sample of known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
The LOQ was calculated as follows:

$$LOQ = 10 (\sigma/S)$$

where S: Slope of the calibration curve, **σ**: Average standard deviation of the response

RESULTS

Chromatographic conditions

In order to separate tadalafil API, phosphate buffer-acetonitrile mixtures were used as the mobile phase. Satisfactory resolution was obtained using the mobile phase system of acetonitrile/phosphate buffer (70:30, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at

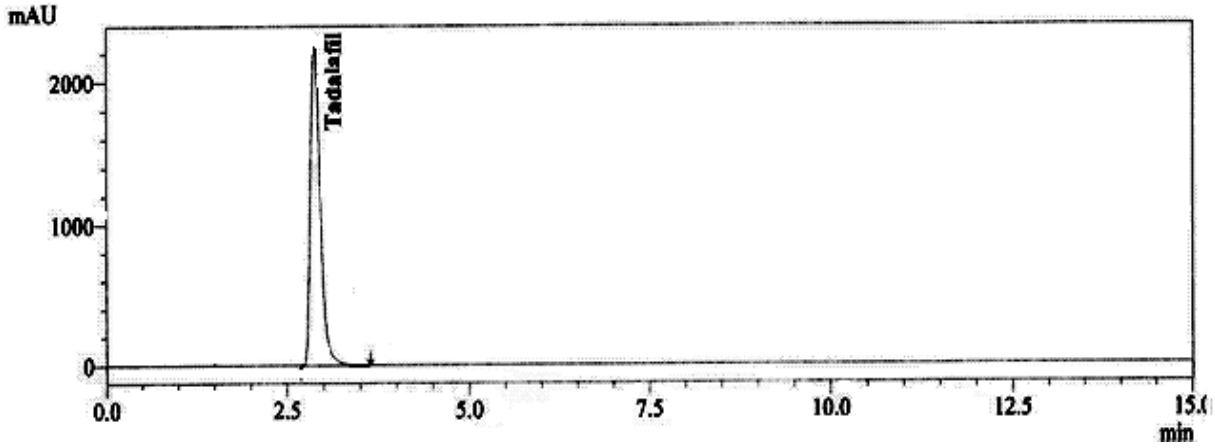

Figure 2. Chromatogram of Tadalafil API.

Table 1. Results of force degradation studies of Tadalafil.

Stress condition/duration/solution	Degradation %
Alkaline degradation (0.005 N NaOH, 1 hr)	10%
Alkaline degradation (0.05 N NaOH, 2 hr)	20%
Oxidative degradation (1 % H_2O_2)	0
Oxidative degradation (3 % H_2O_2)	0
Oxidative degradation (10 % H_2O_2)	0
Oxidative degradation (30 % H_2O_2, 80°C for 10 min)	35
Acidic degradation(0.05 N HCl)	0
Acidic degradation(0.5 N HCl)	0
Acidic degradation(1 N HCl)	15
Thermal degradation (Solid sample, 80°C, 1 hr)	15
Thermal degradation (Solid sample, 100°C, 1 hr)	20
Thermal degradation (Solid sample, 120°C, 1 hr)	26

260 nm. Under these conditions tadalafil with the retention time of tadalafil was 2.88 min. Figure 2 showed a typical chromatogram obtained under these conditions shown.

Forced degradation studies

During the study it was observed that upon treatment of tadalafil with different strengths of base (0.005 N and 0.05 N NaOH), acid (0.05 N, 0.5 N and 1 N HCl) and hydrogen peroxide (30, 10, 3 and 1%) the degradation was observed only with the higher strengths (0.05 N NaOH), acid (1 N HCl) and hydrogen peroxide (30%), where as with the lower strengths of alkali (0.005 N NaOH), acid (0.05 N and 0.05 N HCl) and hydrogen peroxide (1, 3 and 10%) no degradation was observed (Table 1).Further it is important to note that from the chromatograms (Figure 3a to c), it is evident that although the degraded peaks are observed. The tadalafil stable under the applied stress conditions like heat, acid and alkaline and oxidative degradation states.

Linearity

The calibration curve showed good linearity in the range of 50 - 150 µg/ml, for tadalafil API with correlation co-efficient (R^2) of 0.9998 (Figure 4). A typical calibration curve has the regression equation of y = 23646x +568057 for tadalafil.

Precision

The results of system precision (% RSD = 0.26), method precision (% RSD = 0.10) are found within the prescribed limit of ICH guidelines (% RSD < 1%, and % RSD < 2 % respectively in case of system precision and method precision).

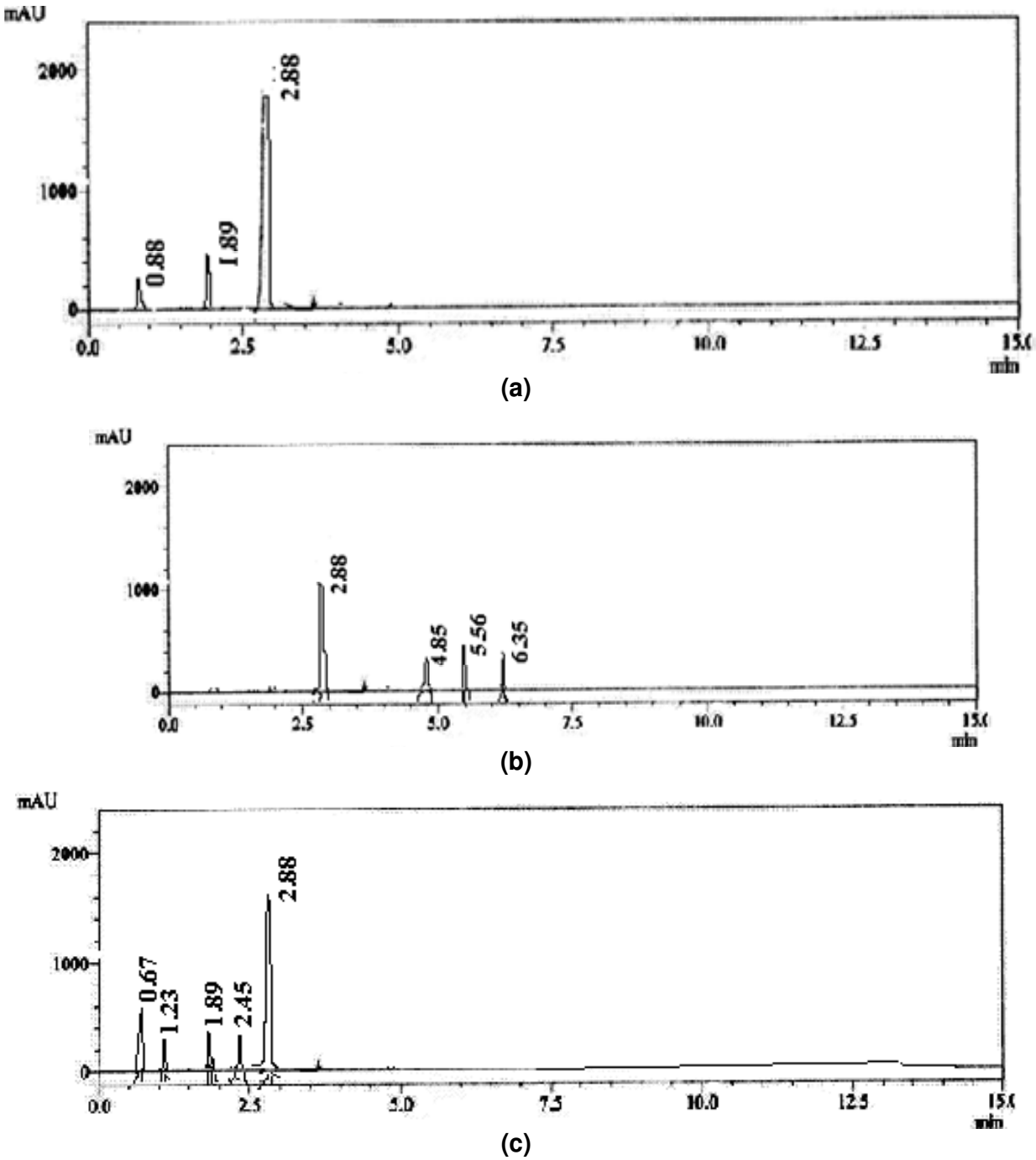

Figure 3. Chromatograms of (a) Base hydrolyzed-degraded sample (b) Thermal degraded sample and (c) Acid hydrolyzed degraded sample.

Intra-assay and Inter-assay

The intra and inter-day variation of the method was carried out and the high values of mean assay and low values of standard deviation and %RSD (%RSD < 2%) within a day and day to day variations for tadalafil revealed that the proposed method is precise in (Table 2 and 3).

Method robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 10%), organic content in mobile phase (± 2%), wavelength of detection (± 5%) and pH of buffer in mobile phase (± 0.2%) studied to determine the robustness of the method are also in favor (Table 4, %RSD < 2%) of the developed RP-HPLC

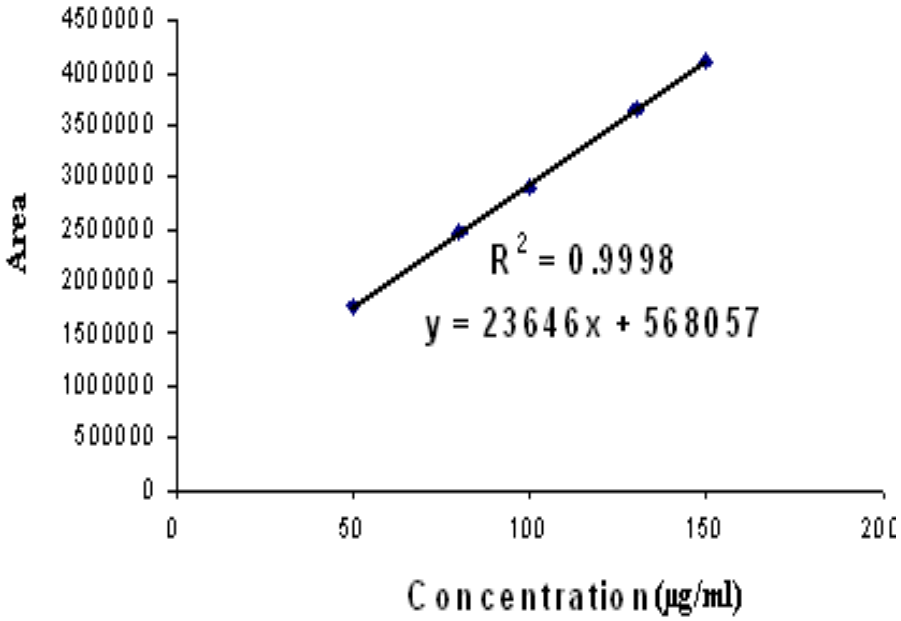

Figure 4. Linearity curve for Tadalafil.

Table 2. Intra-assay precision data of proposed RP-HPLC method (Method Ruggedness).

	Mean (% w/w)	SD	% RSD
Assay-1	99.75	0.450	0.45
Assay-2	99.30	0.230	0.24
Intra assay	99.53	0.410	0.40

Table 3. Inter-assay precision data of proposed RP-HPLC method.

	Mean (% w/w)	SD	% RSD
Assay-1	99.35	0.090	0.08
Assay-2	99.20	0.240	0.26
Intra assay	99.43	0.310	0.20

Table 4. The influence of changes in chromatographic parameters on RP-HPLC analysis of Tadalafil API (Method robustness).

Change in parameter	% RSD
Flow (0.7 ml/min)	0.65
Flow (0.9 ml/min)	0.92
Wavelength (255 nm)	0.74.
Wavelength (265 nm)	0.56
pH (6.8)	0.04
pH (7.2)	0.48
Organic phase composition (-2%)	0.58
Organic phase composition (+2%)	0.80

method for the analysis of tadalafil API.

LOD and LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.05 and 0.5 µg/ml, respectively.

Specificity and stability in analytical solution

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the tadalafil API was stable in solution from up to 24 h at 25°C.

The results of linearity, precision, inter and intra-assays, method robustness, LOD, LOQ and specificity and stability in analytical solution established the validation of the developed RP-HPLC assay for the analysis of tadalafil.

DISCUSSION

The present study is the first time report on stability indicating assay of tadalafil in presence of degradation products by HPLC. In this method isocratic elution method is selected for the analysis of tadalafil API because it gave better base line separation and peak width, which is suitable for the routine analysis of tadalafil. The developed method was validated as per ICH guidelines (ICH, 1996) and its updated international convention (ICH, 2002).

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and enables recommendation of storage conditions, retest periods, and shelf life to be established (Avarez-Lueje et al., 2003; Abdul-Fattah et al., 2002; Lambropouls et al., 1999; Bebawy, 2003). The assay of tadalafil API (Active Pharmaceutical Ingredient) in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines (ICH, 1993) and (USP, 2003).

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