AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

# HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF TADALAFIL IN TABLET DOSAGE FORM

**Satish A. Patel *, Natvarlal J. Patel**

1.Department of Pharmaceutical Quality, Assurance Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India

## ABSTRACT

A simple and sensitive high performance thin layer chromatography (HPTLC) method has been developed for the quantitative estimation of Tadalafil in its single component tablet formulation (20 mg). Tadalafil was chromatographed on silica gel 60 $F_{254}$ TLC plate using chloroform: methanol (9:1, v/v) as mobile phase. Tadalafil showed Rf value 0.78 $\pm$ 0.008 and scanned at 285 nm using a camag TLC scanner 3. The method was validated in terms of linearity (100 – 800 ng/spot), precision (intra-day variation, 0.38 to 0.81% and inter-day variation, 0.45 to 1.90%), accuracy (100.3 ± 0.76) and specificity. The limit of detection and limit of quantification for Tadalafil were found to be 28.11 ng/spot and 93.45 ng/spot, respectively. The developed method was successfully used for the assay of Tadalafil tablet formulation. The method was found to be simple, sensitive, specific, accurate and precise and can be used for the routine quality control testing of Tadalafil in tablet dosage form.

**Keywords:** Tadalafil, HPTLC, validation, tablet

*Corresponding Author Email: satishpatel_77@yahoo.com

Received 11 September 2011, Accepted 16 September 2011

Please cite this article in press as: Patel S *et al.*, High Performance Thin Layer Chromatographic method for Determination of Tadalafil in tablet dosage form. American Journal of PharmTech Research 2011.

## INTRODUCTION

Tadalafil is chemically, (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[1]. Tadalafil is a phosphodiesterase type 5-inhibitor, used in the management of erectile dysfunction[2]. Tadalafil is not official in any of the pharmacopoeias. The literature survey reveals HPLC[3,4,5,6,7], LC/MS[8,9], capillary electrophoresis[10] and ESI-MS-MS[11] methods for the determination of Tadalafil pharmaceutical dosage forms as well as in biological fluids. The literature survey does not reveal any simple HPTLC method for the determination of Tadalafil in tablet dosage form. The present manuscript describes simple, sensitive, accurate, precise and specific HPTLC method for the estimation of Tadalafil in tablet.

## MATERIALS AND METHODS

### Apparatus

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

### Reagents and Materials

Pharmaceutical grade of Tadalafil was kindly supplied as a gift sample from Zydus Cadila Healthcare Ltd., Gujarat, India. Silica Gel 60 $F_{254}$ TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. The pharmaceutical tablet formulation containing 20 mg of Tadalafil was procured from the local pharmacy. Chloroform, methanol and acetonitrile (HPLC grade, Rankem, India) were used for mobile phase preparation and as solvents.

### Preparation of standard solution

A standard solution of Tadalafil (100 µg/ml) was prepared by accurately weighing Tadalafil (10 mg) and transferred in 100 ml volumetric flask, dissolved in and diluted up to mark with acetonitrile.

### Preparation of sample solution

Twenty tablets were weighed, their average weight was determined, and crushed in mortar. Powder equivalent to 10 mg of Tadalafil was weighed and transferred to 100 ml volumetric flask. The drug from powder were dissolved and extracted with acetonitrile. To ensure complete extraction of drugs it was sonicated for 30 min. The extract was filtered through Whatman filter

paper No. 41 and residue was washed with acetonitrile. The extract and washing were pooled and transferred to another 100 ml volumetric flask and volume was made with acetonitrile to achieve final concentration of 100 µg/ml of Tadalafil. Five microlitres of this solution was applied to the HPTLC plate to get 500 ng/spot and followed by development.

**Chromatographic conditions**

The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 $F_{254}$ aluminum sheets (10 × 10 cm) (pre-washed with methanol and dried in air); mobile phase, chloroform: methanol (9:1, v/v); chamber saturation time, 30 min; temperature, 25 ± 2°, migration distance, 80 mm; wavelength of detection, 285 nm; slit dimensions, 5 × 0.45 mm; scanning speed, 10 mm/s. Following spotting parameter were used - band width, 6 mm; distance from the plate edge, 10 mm; space between two bands, 10 mm and spraying rate, 1 µl/s.

**Chromatographic separation**

Six microlitres of standard solution of Tadalafil (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of chloroform: methanol (9:1, v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 285 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

**Validation of the proposed method**

The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines[12].

**Linearity (Calibration curve)**

Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml of standard Tadalafil solution (100 µg/ml) were spotted on precoated TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. Each concentration was spotted five times on the TLC plate. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

**Accuracy (% Recovery)**

The accuracy of the method was determined by calculating recoveries of Tadalafil by the standard addition method. Known amounts of standard solutions of Tadalafil was added at 50,

100 and 150 % level to prequantified sample solutions of Tadalafil (300 ng/spot). The amount of Tadalafil was estimated by applying obtained values to the regression line equation.

**Method Precision (% Repeatability)**

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of Tadalafil (500 ng/spot) without changing the parameters of the proposed method.

**Intermediate Precision (Reproducibility)**

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solution of Tadalafil (300, 400 and 500 ng/spot) for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

**Limit of detection (LOD) and limit of quantification (LOQ)**

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines[12].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma /S$

Where $\sigma$ = Standard deviation of the response

S = Slope of calibration curve

**Specificity**

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for deflazacort in the samples were confirmed by comparing the $R_f$ and spectra of the spots with that of the standards.

**Analysis of Tadalafil in tablet**

Five microlitres of sample solution was applied to the TLC plate to get 500 ng/spot and followed by development and scanning as described earlier. Analysis was carried out in triplicate, peak areas were measured at 285 nm and sample concentrations calculated. The amount of Tadalafil present in the sample solution was determined by fitting area values of peak corresponding to Tadalafil into the equation of line representing calibration curve of Tadalafil. The potential interference from excipients was also examined.

## RESULTS AND DISSCUSION

Tadalafil is soluble in acetonitrile; therefore acetonitrile was selected as solvent. Several mobile phases were tried to accomplish good separation of Tadalafil. Using the mobile phase

chloroform: methanol (9:1, v/v) and 10 × 10 cm silica gel $60F_{254}$ aluminum-backed plates, good separation was attained with retardation factor ($R_f$) values of 0.78 ± 0.008 for Tadalafil (Figure 1 and 2). A wavelength of 285 nm was used for quantification of the drug. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution.

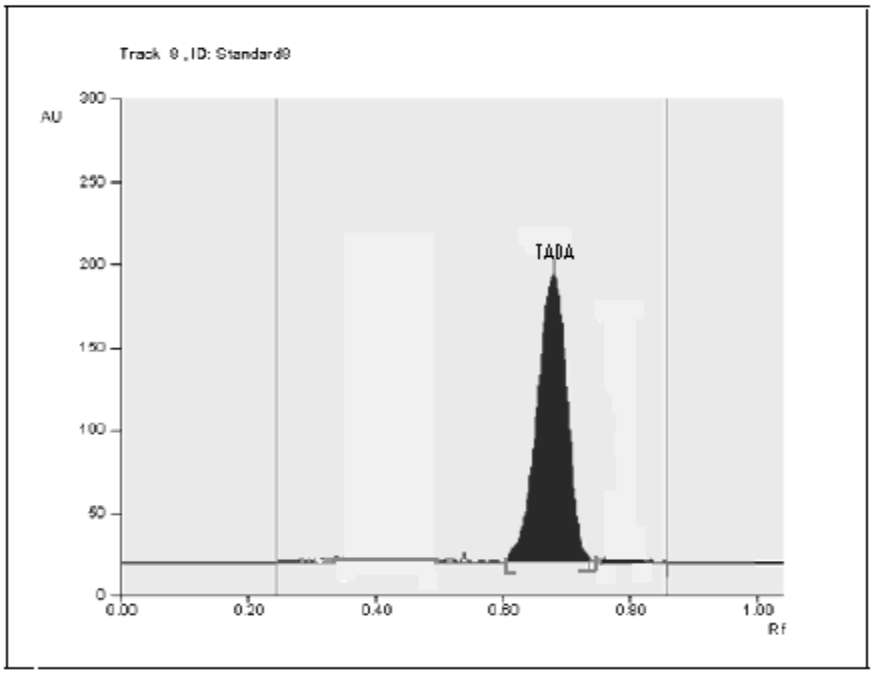


**Figure 1: HPTLC chromatogram of tadalafil with corresponding Rf value at 285 nm**

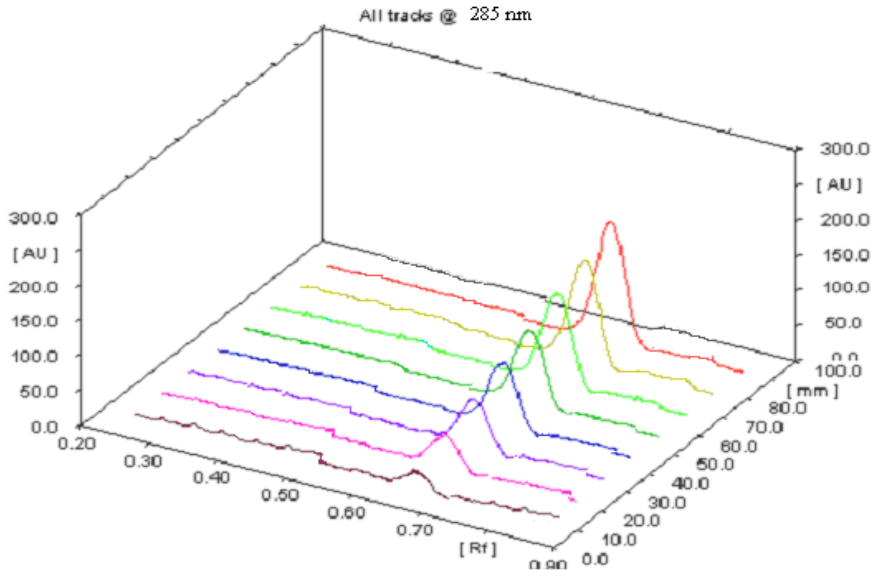


**Figure 2: 3D-Chromatogram show peaks of Tadalafil in different concentrations at 285 nm**

Linearity range for Tadalafil was found in the concentration range of 100 to 800 ng/spot, with a correlation coefficient of 0.9970. The average linear regression equation was represented as Y = 2.351X + 68.996, where X = concentration of Tadalafil in ng/spot and Y = peak area. The limit of detection and limit of quantification for Tadalafil were found to be 28.11 ng/spot and 93.45 ng/spot, respectively indicate sensitivity of the method.

The intra-day precision (% RSD) was calculated for standard Tadalafil solutions (300, 400 and 500 ng/spot) for 3 times on the same day. The inter-day precision (% RSD) was calculated for standard Tadalafil solutions (300, 400 and 500 ng/spot) for 3 times over a period of one week. The intra-day and inter-day variation (% RSD) were found to be in the range of 0.38-0.81 and 0.45-1.90, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (500 ng/spot) of Tadalafil six times without changing position of the plate and % RSD for measurement of peak area was found to be 0.24. The % RSD for measurement of peak area ensures proper functioning of HPTLC system indicates repeatability of the proposed method. Different validation parameters for the proposed HPTLC method for determining Tadalafil content are summarized in Table 1.

**Table 1: Regression Analysis Data and Summary of Validation Parameters for Proposed HPTLC Method**

| Parameters | Results |
|---|---|
| Linearity range (ng/spot) | 100 - 800 |
| Slope | 2.351 |
| Intercept | 68.996 |
| Correlation co-efficient ($r^2$) | 0.9970 |
| Precision (% RSD) | |
| Intra-day (n = 3) | 0.38 - 0.81 |
| Inter-day (n = 3) | 0.45 - 1.90 |
| Repeatability of peak area (% RSD) (n = 6) | 0.24 |
| Accuracy (% Recovery) (n = 5) | 100.3 ± 0.76 |
| Limit of detection (LOD) (ng/spot) | 28.11 |
| Limit of quantification (LOQ) (ng/spot) | 93.45 |
| Specificity | Specific |

n is number of determination and RSD is relative standard deviation.

Accuracy of the method was evaluated by calculating recovery of Tadalafil by standard addition method at 3 different levels of the calibration curve (n = 5). The % mean recovery was found to be 100.3 ± 0.76 ensuring that the method is accurate (Table 2).

**Table 2: Recovery data for the Proposed Method**

| Drug | Level | Amount of sample taken (ng/spot) | Amount of standard spiked (%) | Mean % Recovery ± S. D. (n = 5) |
|---|---|---|---|---|
| TADALAFIL | I | 300 | 50 % | 100.7 ± 0.84 |
| | II | 300 | 100 % | 100.3 ± 0.80 |
| | III | 300 | 150 % | 99.85 ± 0.65 |

n is number of determination and S.D. is standard deviation.

The method was found to be specific for Tadalafil. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for Tadalafil in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. The peak purity of Tadalafil was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of spot. Good correlation was also found between standards and sample spectra (Figure 3). None of the formulation excipients were interferes in the quantification of Tadalafil at this Rf value.

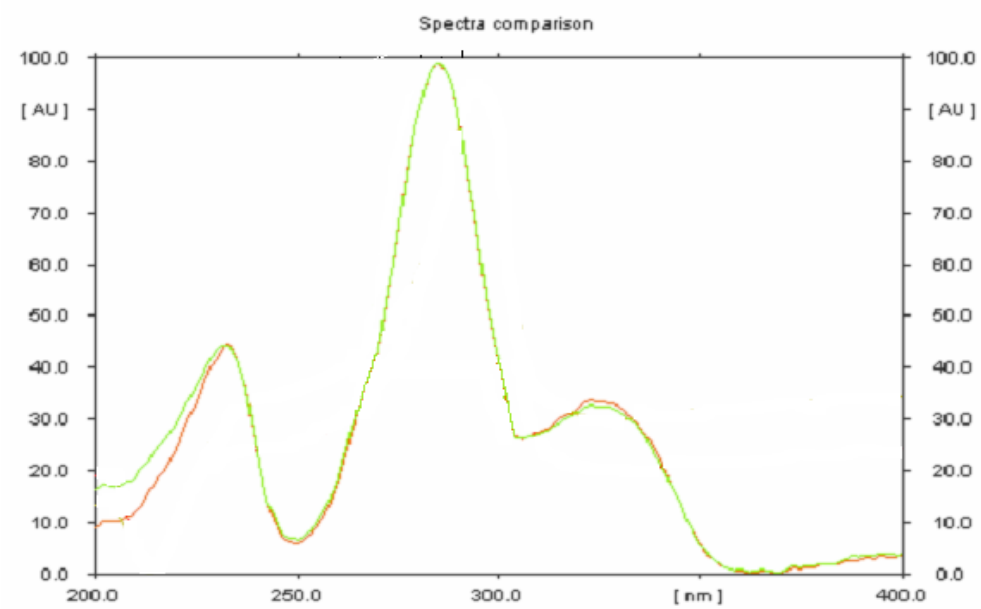


**Figure 3: Overlain UV absorption spectrum of standard and sample Tadalafil**

This method was applied to determine the content of Tadalafil in market sample of single component Tadalafil tablet. The average percentage of Tadalafil in market sample was found to be 99.98 ± 0.75 (n = 6). The results are in agreement with the labeled value of Tadalafil in tablet dosage form (Table 3). The results indicate that the proposed HPTLC method was found to be simple, sensitive, specific, precise and accurate for the estimation of Tadalafil in tablet formulations.

**Table 3: Analysis of Tablet Formulation of Tadalafil by Proposed HPTLC Method (n = 6)**

| Tablet | Label claim (mg) | Parameters | % amount found (n = 6) HPTLC method |
|---|---|---|---|
| Brand A | 20 | Mean | 100.7 |
| | | S. D. | 1.04 |
| Brand B | 20 | Mean | 99.25 |
| | | S. D. | 0.46 |

n is number of determination and S.D. is standard deviation.

## CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Tadalafil. The observations and results obtained from this study, including specificity, linearity and range, accuracy, precision (method precision as repeatability and intermediate precision as intra and inter day precision) are lie well within acceptable results. From the experimental studies it can be concluded that proposed method can be adopted for the routine analysis of Tadalafil in tablets without interference of excipients.

## ACKNOWLEDGEMENT

The author is thankful to Zydus Cadila Healthcare Ltd., Gujarat, India for providing the gift sample of Tadalafil and Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University for providing all the facilities to carry out the research work.

## REFERENCES

1. Maryadele JO Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals. 14th ed., Whitehouse station, New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc; 2006: 1550.
2. Sweetman SC. Martindale: The Complete Drug Reference, 35th ed., London, UK: Pharmaceutical Press; 2007: 1990.
3. Hassan Y, Aboul E, Ali I. Determination of Tadalafil in pharmaceutical preparation by HPLC using monolithic silica column. Talanta 2005; 65: 276-280.
4. Rabbaa-Khabbaz L, Abi Daoud R. A sensitive and simple high performance liquid chromatographic method for quantification of Tadalafil in human serum. J Appl Res 2006; 6: 170-175.

5. Ching-Ling C, Chen-Hsi C. Determination of Tadalafil in small volumes of plasma by high-performance liquid chromatography with UV detection. J Chromatogr B 2005; 822: 278-284.
6. Shakya AK, Abu-Awwad AN, Arafat TA, Munther M. Validated liquid chromatographic-ultraviolet method for the quantitation of Tadalafil in human plasma using liquid-liquid extraction. J Chromatogr B 2007; 852: 403-408.
7. Subba Rao DV, Radhakrishnan P, Himabindu V. Development of a validated LC assay for Tadalafil bulk drug and pharmaceutical dosage form. Chromatographia 2008; 67: 183-188.
8. Ramakrishna NVS, Vishwottam KN, Puran S, Koteshwara M, Manoj S, Santosh M, Chidambara J, Wishu S, Sumatha B. Quantitation of Tadalafil in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionization. J Chromatogr B 2004; 809: 243-249.
9. Xiaolan Z, Xiao X, Song S, Chen B, Zhang F, Shouzhuo Y, Zutian Z, Dajin D, Hongwei H. Simultaneous determination of sildenafil, vardenafil and Tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A 2005; 1066: 89-95.
10. Ali I, Aboul-Enein HY. Validated method for Tadalafil analysis in pharmaceutical preparations by capillary electrophoresis. Chromatographia 2004; 60: 187-191.
11. Abdel-Hamid ME. Determination of sildenafil, Tadalafil and vardenafil in tablets and adulterated herbal products by ESI-MS-MS. J Liq Chromatogr Rel Technol 2006; 29: 591-603.
12. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology: 2005.