FORCED DEGRADATION STUDIES, QUANTIFICATION AND *IN-VITRO* DISSOLUTION STUDIES OF TADALAFIL BY SPECTROFLUORIMETRY

KAVITHA A[1], VIJAYA DURGA D[1], HIMA BINDU S[1], ESHVENDAR K[2], KHALEEL N[3], PANI KUMAR D ANUMOLU[1*]

[1]Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Andhra Pradesh-500090,[2] NIPER, Balanagar, Hyderabad-500037,[3] Aizant Drug Research Solutions Pvt Ltd, Analytical R&D, Hyderabad-500014,
E-mail: panindrapharma@yahoo.co.in.

ABSTRACT

Objective: In the present study a simple and sensitive spectrofluorimetric method has been developed and validated for the estimation of tadalafil in pure and pharmaceutical dosage forms including its *in-vitro* dissolution studies and forced degradation studies. **Methods:** Fluorescence intensity of tadalafil measured at an emission wavelength (λ_{em}) of 676 nm after excitation at 292 nm in dimethyl sulphoxide as solvent by using spectrofluorimeter RF-5301 PC instrument. **Results:** Linearity was obeyed in the range of 0.1-2.0 µg/mL with good correlation coefficient of 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) for this method are 0.074 and 0.22 µg/mL respectively. The developed method was statistically validated as per ICH guidelines. The percentage relative standard deviation values were found to be less than 2 for accuracy and precision studies. The results obtained were in good agreement with the labeled amounts of the marketed formulations. **Conclusion:** The proposed method is simple, sensitive and specific. Hence it can be applied for quantification of tadalafil in pharmaceutical dosage forms.

Keywords: Tadalafil, Spectrofluorimetry, Validation

INTRODUCTION

Tadalafil, which is chemically (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxy- phenyl) pyrazino[20 ,10:6,1] pyrido [3,4-b]indole-1,4-dione (Figure 1), is an orally administered drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension [1].

A detailed literature survey for tadalafil revealed that several analytical methods are reported for the determination of tadalafil by spectrophotometry [2], high performance liquid chromatography [3-5], high performance thin layer chromatography [6], gas chromatography-mass spectroscopy [7] and colorimetry [8]. Moreover, tadalafil has not been reported by stability indicating spectrofluorimetric method development and validation including dissolution studies.

Spectrofluorimetry [9-14] has assumed a major role in drug analysis because of its greater sensitivity and selectivity than absorption spectrophotometry. Therefore, the aim of present work was to develop and validate a spectrofluorimetric method for the quantification of tadalafil including its degradation studies and dissolution studies.
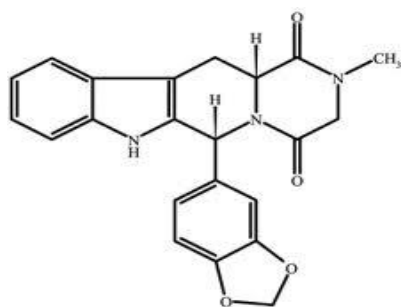

Figure 1: Chemical structure of tadalafil

MATERIALS AND METHODS

Materials

Tadalafil standard gift sample was provided by Mylan Pharma Ltd, Hyderabad, India. The commercially available tablet dosage forms (Tazzle 10 and Megalis 20). The analytical grade dimethyl sulphoxide (DMSO), ethanol, methanol and acetonitrile were purchased from Sd fine chemicals, Mumbai.

Instrument

The fluorescence spectra and measurements were recorded using Shimadzu RF-5301 PC Spectrofluorophotometer, equipped with 150 watt Xenon arc lamp, 1cm quartz cell was used, connected to RFPC software. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm.

Selection of solvent

For spectrofluorimetric method purpose, various solvents were investigated such as acetonitrile, methanol, DMSO, and ethanol. The fluorescence intensity of tadalafil was high in DMSO than other solvents and no fluorescence intensity observed in water, acetic acid and dimethyl formamide. Finally, we selected DMSO as solvent for quantification, forced degradation studies and *in-vitro* dissolution studies of tadalafil.

Preparation of standard solution of tadalafil

The standard stock solution (1mg/mL) tadalafil was prepared by transferring 10 mg of tadalafil in 10 mL volumetric flask and volume was made up to the mark with dimethyl sulphoxide (DMSO).

Procedure for pharmaceutical preparation

Twenty tablets of each marketed formulation such as Tazzle-10 (10mg of Tadalafil), Megalis 20 (20mg of Tadalafil) were taken and accurately weighed. Average weight was determined and crushed to fine powder. An accurately weighed quantity of equivalent powder was transferred in to a 10 mL capacity of volumetric flask containing few mL of DMSO and sonicated for 15 min and made up to the mark with DMSO. The above solution was filtered through whatmann filter paper and filtrate was again diluted to get a final concentration of 1.2µg/mL in all solutions. All determinations were conducted in triplicate and the emission spectrum was recorded.

Method Validation

The method was validated as per ICH guidelines [16] for linearity, accuracy, precision, specificity; limit of detection and limit of quantification.

Linearity

To determine linearity, aliquots of tadalafil stock solutions were taken into 10mL volumetric flasks and diluted up to the mark with the solvent such that the final concentration of tadalafil was in the range of 0.1-2.0 µg/mL and analyzed them by spectrofluorimeter with the proposed method. The calibration curve was constructed by plotting the analyte fluorescence intensity against the concentration (µg/mL). Calibration curve was evaluated by its correlation coefficient.

Accuracy (Recovery studies)

The accuracy was carried out by recovery studies using standard addition method; known amount of standard drug was added to pre analyzed sample of tadalafil in according to 80, 100 and 120% levels of labeled claim and then subjected to the proposed method. The experiment was conducted in triplicate. The percentage recovery and percentage relative standard deviation (% RSD) were calculated for each concentration.

Precision

Precision of the method was determined by intra-day precision and inter-day precision variations as per ICH guidelines. For both intra-day precision and inter-day precision of the samples containing tadalafil 0.8,1.6, and 2.0 µg /mL were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter day precision) . The % RSD was calculated.

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

Applications of proposed method

Dissolution studies

The dissolution rate studies of tadalafil from tablets were performed on a paddle-stirrer type of apparatus. The developed method was used to analyze samples after *in-vitro* dissolution of tadalafil under standard conditions specified for single entity products by FDA [16, 17]. The in-vitro dissolution was performed in 1000 mL of dissolution medium of 0.5% sodium lauryl sulphate for 45min at a temperature of 37±0.5⁰C by USP paddle type apparatus with 50 rpm rotation speed. In order to obtain the dissolution profile, the cumulative percentage of drug release was plotted against time intervals (min).

Forced degradation studies

Stock solution of tadalafil (1 mg/mL) was transferred into a series of 10 mL volumetric flasks to obtain a final concentration of 10 µg/mL by adding 0.1M hydrochloric acid (Acid hydrolysis), 0.1M sodium hydroxide (Alkali hydrolysis), 3% hydrogen peroxide (Oxidation), temperature effect and photo degradation were studied by placing 10 µg/mL sample solution in hot air oven at 105⁰C and in UV chamber at 254nm respectively. These solutions were kept at specified conditions for 24hrs. Aliquot volumes of degraded solutions were transferred to a series of 10 mL volumetric flasks and neutralized with 0.1M hydrochloric acid, 0.1M sodium hydroxide for alkali hydrolysis and acid hydrolysis respectively. Finally, the plot between percent of tadalafil degraded against time interval was constructed.

RESULTS AND DISCUSSION

In order to develop spectrofluorimetric method for quantification of tadalafil, various solvent systems were investigated such as methanol, ethanol, dimethyl sulphoxide (DMSO), dimethyl formamide, acetonitrile and glacial acetic acid (Figure 2). The fluorescence intensity of tadalafil was maximum or high in DMSO than other solvents. Hence, we selected DMSO as solvent for quantification of tadalafil at excitation wavelength 292 nm and emission wavelength 676nm (Figure 3).
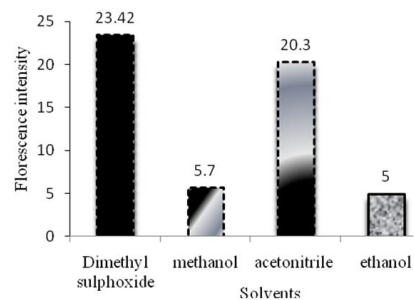

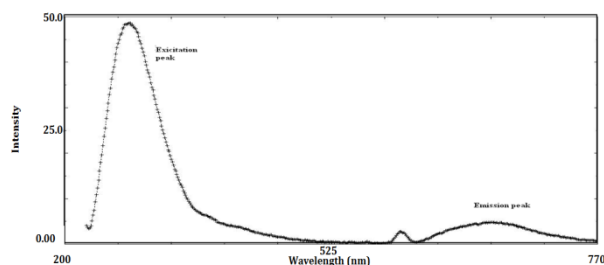**Figure 2: Effect of solvents on fluorescence intensity of tadalafil**

Figure 3: Excitation and emission peaks of tadalafil

The calibration curve was linear in the range of 0.1-2.0 µg/mL with a correlation coefficient of 0.999 (Figure 4). Limit of detection (LOD) and Limit of quantification (LOQ) were experimentally verified to be 0.074 and 0.22 µg /mL respectively, which indicates that the method shows high sensitivity. Optimized conditions for proposed method were given in Table 1.
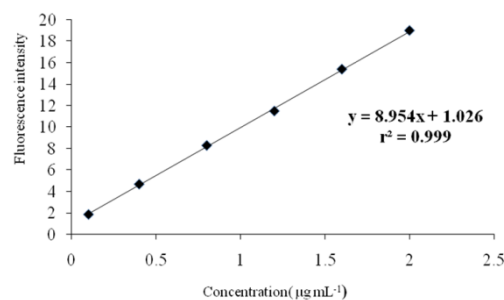

$$y = 8.954x + 1.026$$
$$r^2 = 0.999$$

Figure 4: Linearity curve

Table 1: Optimum parameters for proposed method

Parameter	Value
$\lambda_{exitation(nm)}$	292
$\lambda_{emission(nm)}$	676
Regression equation (Y)	8.95x+1.02
Intercept	1.02
Slope	8.95
LOD (µg/ mL)	0.074
LOQ (µg /mL)	0.22

Table 2: Accuracy (Recovery) studies

Brand name	Recovery level %	Theoritical content(mg)	Amount found(mg) ± SD (n=3)	%Recovered	RSD (%)	SEM
Tazzle-10	80	18	17.9±0.04	99.4	0.22	0.02
	100	20	19.8±0.02	99	0.30	0.011
	120	22	22.1±0.02	100.4	0.30	0.011
Megalis-20	80	18	17.8±0.03	98.8	0.16	0.017
	100	20	19.9±0.02	99.5	0.15	0.011
	120	22	21.8±0.03	99	0.16	0.017

Table 3: Precision values

Concentration (µg /mL)	Intraday Precision (n=6)		Inter-day Precision (n=6)	
	Amount found (mg) ±SD	%RSD	Amount found (mg) ±SD	%RSD
	0.81 ± 0.01	1.23	0.80 ±0.02	1.05
1.6	1.59 ±0.02	1.05	1.01 ±0.02	1.06
2.0	1.98 ±0.02	0.62	2.1 ±0.02	1.32

Table 4: Estimation of amount present in tablet dosage form

Brand name	Label claim per tablet(mg)	Amount of Mean drug found (mg) ± SD (n=3)	RSD (%)	SEM	% Drug estimated
Tazzle-10	10	9.9± 0.02	0.2	1.06	99.5
Megalis-20	20	20.10± 0.03	0.3	0.62	100.2

Dissolution studies of two tadalafil tablets (Tazzle 10 and Megalis 20) conducted by proposed spectrofluorimetric method reveal that more than 90% of labeled amount is dissolved in 45min, which is correlating with FDA guidelines (Figure 5). The stability of tadalafil was studied in terms of fluorescence intensity in the presence of acid, alkaline, oxidative agent, photo and thermal degradation and constructed a plot between amount of drug degraded in terms of (%) Vs time interval (min). The % degradation of tadalafil increases with time in the presence of alkaline (0.1M NaOH), acid hydrolysis (0.1M HCl), oxidation (3% H_2O_2) and thermal degradation studies (105°C) but no significant difference observed in the presence of photo degradation (254 nm). It reveals that tadalafil was found to be susceptible to alkali, acid, oxidation and thermal degradation (Figure 6).
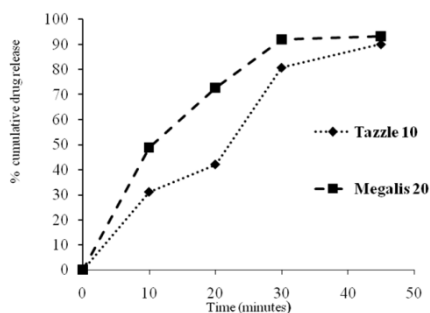

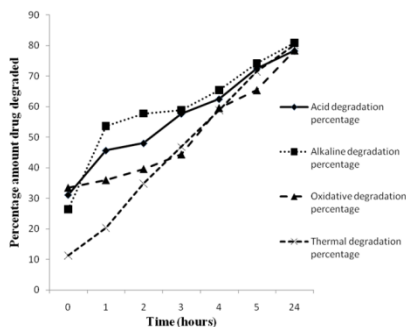**Figure 5: Dissolution profiles of tadalafil tablet dosage forms**.

Figure 6: Degradation studies of tadalafil

CONCLUSION

It is concluded that the proposed method was found to be simple, accurate and precise for the quantification of tadalafil in tablet dosage forms and its *in-vitro* dissolution samples. The assay values were in good agreement with their respective labeled claim. This spectrofluorimetric method has been found to be better because of its specificity, sensitivity, readily available solvent and also utilized for forced degradation studies and *in-vitro* dissolution studies. These advantages encourage that the proposed method can be routinely employed in quality control for analysis of tadalafil in tablet dosage forms and dissolution studies.

REFERENCES

1. CIMS, Current index of medical specialities. Bangalore: CMP Media India Pvt Ltd.; 2012.
2. Mohammad Yunoos,Gowri Sankar D, Pragati Kumar B and Shahul Hameeduv, Spectrophotometric method for the estimation of tadalafil in bulk and tablet dosage form. E-Journal of Chemistry 2010; 7(3): 833-36.
3. Prasanna Reddy B, Amarnadh Reddy K, and Reddy MS.Validation and stability indicating RP-HPLC method for the determination of tadalafil API in pharmaceutical formulations. Research in Pharmaceutical Biotechnology 2010; 2(1):1-6.
4. Kannappan N, Deepthi Y, Divya Y, Shashikanth, R and Mannavalan, Method development and validation of stability indicating methods for assay of tadalafil and sildenafil citrate by high performance liquid chromatrography. International Journal of ChemTech Research 2010; 2(1): 329-33.
5. Sutar AS, Magduma S, Patila S and Naikawadia NS. RP-HPLC estimation of tadalafil in tablet dosage form. Int. J. Chem. Sci 2008; 6(2): 1223-27.
6. Satish A and Natvarlal J. High performance thin layer chromatographic method for determination of tadalafil in tablet dosage form. American Journal of PharmTech Research 2011;1(3): 138-146.
7. Nikolou P, Papoutsis I, Athanaselis S, Alevisopoulos G, Khraiwesh A, Pistos C, and Spiliopoulou C. Development and validation of a GC/MS method for the determination of tadalafil in whole blood. Journal of pharmaceutical and Biomedical Analysis 2011; 56 (3):577-581.

8. Raju GHU, Ganapathy Sankar DG and Naidu PY. New UV visible spectrophotometric estimation of tadalafil in bulk and pharmaceutical preparations. Asian journal of research in chemistry 2010; 3(4).
9. Rasha A, Shaalan T and Belol S. Simultaneous spectrofluorimetric determination of amlodipine besylate and valsartan in their combined tablets. Drug testing and analysis 2010; 2: 489-493.
10. Mohamed W, Fathallah FB and Nahed ME. Synchronous fluorescence spectrofluorimetric method for the simultaneous determination of metaprolol and felodipine in combined pharmaceutical preparation. Chemistry central J 2011; 5(70).
11. El-Wasseef DR, El-Sherbiny DT, Abu-El-Enein MA and El-Ashry SM. Simultaneous determination of labetalol and furosemide by first-derivative synchronous spectrofluorimetry. J Floresec 2009;19:817-828.
12. Fathalla B, El-Brashy A and Nahed El-Enany. Conventional and first derivative synchronous fluorometric determination of ethamsylate in pharmaceutical preparations and biological fluids, application to stability studies. J Floresec 2011; (21): 1371-84.

13. Fawzia I, Mohie KS, Manal I, and Mary EK. Validated stability-indicating spectrofluorimetric methods for the determination of ebastine in pharmaceutical preparations. Chemistry central J 2011; 5 (11).
14. Mesut A and Emrah k. Validated spectrofluorimetric method for the determination of sunitinab malate, dye complexation approach for a novel anticancer drug. Acta pharmaceutica sciencia 2010; 52: 469-485.
15. International Conference on Harmonization, ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2B, 1996.
16. FDA Guidance for industry: Immediate release solid dosage forms: In-vitro dissolution testing and in-vivo bioequivalence documentation. Rockville (MD): Food and Drug Administration; 1995.
17. Panikumar AD, Venkat raj Y, Sunitha G, Sathesh babu PR, Subrahmanyam CVS.Development of biorelevant and discriminating method for dissolution of efavirenz and its formulations. Asian J Pharm Clin Res 2012; 5 suppl 3:1-4.