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

KAVITHA A¹, VIJAYA DURGA D¹, HIMAA BINDU S¹, ESHVENDAR K², KHALEEL N³, PANI KUMAR D ANUMOLU¹*  
¹Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Andhra Pradesh-500090, ²NIPER, Balanagar, Hyderabad-500037, ³Aizant Drug Research Solutions Pvt Ltd, Analytical R&D, Hyderabad-500014,  
E-mail: panindrpharma@yahoo.co.in.

Received: 29 March 2013, Revised and Accepted:

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 (λₑ) of 676 nm after excitation at 292 nm in dimethyl sulfoxide as solvent by using spectrofluorimeter RF-S301 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[2,1-c][1,2,3]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](image)

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-S301 PC Spectrofluorophotometer, equipped with 150 watt Xenon arc lamp, 1 cm 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 (10 mg of Tadalafil), Megalis 20 (20 mg 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 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±0.5°C 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 105°C 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 sulfoxide (DMSO), dimethyl formamide, acetone 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).

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.

![Figure 2: Effect of solvents on fluorescence intensity of tadalafil](image)

![Figure 3: Excitation and emission peaks of tadalafil](image)

![Figure 4: Linearity curve](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{ex}$ (nm)</td>
<td>292</td>
</tr>
<tr>
<td>$\lambda_{em}$ (nm)</td>
<td>676</td>
</tr>
<tr>
<td>Regression equation (Y)</td>
<td>8.95x+1.02</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.02</td>
</tr>
<tr>
<td>Slope</td>
<td>8.95</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.074</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.22</td>
</tr>
</tbody>
</table>
2. uv, Spectrophotometric method for the assay of tadalafil and sildenafil citrate


susceptible to alkali, acid, oxidation and thermal degradation (Figure 2). It revealed that tadalafil was found to be susceptible to alkali, acid, oxidation and thermal degradation (Figure 6).

Table 2: Accuracy (Recovery) studies

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

Table 3: Precision values

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intraday Precision (n=6)</th>
<th>Inter-day Precision (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found (mg) ± SD</td>
<td>%RSD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>0.81±0.01</td>
<td>1.23</td>
</tr>
<tr>
<td>2.0</td>
<td>1.59±0.02</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Table 4: Estimation of amount present in tablet dosage form

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Label claim per tablet (mg)</th>
<th>Amount of drug found (mg) ± SD (n=3)</th>
<th>RSD (%)</th>
<th>SEM</th>
<th>% Drug estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tazzle-10</td>
<td>10</td>
<td>9.9±0.02</td>
<td>0.2</td>
<td>1.06</td>
<td>99.5</td>
</tr>
<tr>
<td>Megalis-20</td>
<td>20</td>
<td>20.10±0.03</td>
<td>0.3</td>
<td>0.62</td>
<td>100.2</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENT

The authors are thankful to the management and Prof.C.V.S.Subrahmanyam, Principal, Gokaraju Rangaraju College of Pharmacy.

REFERENCES