Stability-indicating methods for determination of tadalafil in presence of its degradation product

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ABSTRACT

Four different stability indicating assay methods were developed and validated for the determination of tadalafil in the presence of its degradation product. The first and second method was based on the derivative and derivative ratio spectrophotometric technique using mixture of acetonitrile and water (1:1) as a solvent. In the third method, we used TLC-densitometric technique using high performance thin-layer chromatography plates with a developing system consisting of chloroform: acetone: ammonia (9:1:0.1, by volume). The fourth method was a high performance liquid chromatography. Separation of tadalafil from its degrade using C_{18} column and a mobile phase consisting of water: acetonitrile: methanolin the ratio of (45:35:20) at ambient temperature was achieved.

The developed methods were successfully applied to the analysis of pharmaceutical formulations containing tadalafil with excellent recoveries.

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INTRODUCTION

Tadalafil (TD), is (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. (Figure 1)[1]

It is phosphodiesterase-5 inhibitors used for erectile dysfunction. Tadalafil is 61% eliminated mainly in the faeces, 36% eliminated in urine[3]. The literature has analytical methods for quantitative estimation of tadalafil, in pharmaceutical formulations by HPLC[3-6], LC[7-10], NMR[11] and Densitometric determination[12,13].

The present work aimed to develop feasible, sensitive and specific analytical procedures for the analysis of tadalafil in presence of its degradation products.

Adaptation of the proposed procedures to the analysis of the available dosage forms was also an important task in order to solve problems encountered in quality control and analysis of expired samples.

EXPERIMENTAL

Samples

- Tadalafil powder was kindly supplied by Lilly Company, Egypt.
- Pharmaceutical formulation Cialis® tablets (labeled to contain 20 mg of tadalafil), manufactured by Lilly Del Caribe Inc., Puerto Rico industrial Park, Carolina, Puerto Rico.
Reagents

The materials used were chloroform, ammonia, hydrochloric acid (Adwic), acetone (Sigma); Purified water was prepared using a Millipore Milli-Q water purification system, all were of analytical grade. Acetonitrile, methanol (Merck) all were of HPLC grade, all Chemical were purchased from local market (Cairo, Egypt).

Standard solutions

Tadalafil standard and degrade solution (250\(\mu\)g\(\text{mL}^{-1}\)) in acetonitrile water mixture (1:1).

Apparatus

- A double beam UV-vis spectrophotometer (SHIMADZU, Japan) Model UV-1601 PC connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength scanning speed was 2800 nm min\(^{-1}\).
- Pre-coated TLC-plates, silica gel 60 F\(_{254}\) (20 cm \(\times\) 20 cm, 0.2 mm) Fluka, (Switzerland).
- Camag TLC-scanner 3 S/N 130319 with winCATS software.
- Camag Linomat 5 auto sampler (Switzerland).
- Camag micro syringe (100 \(\mu\)L) (Switzerland).
- Liquid chromatograph consists of a “La-Chrom” HPLC instrument (Hitachi-Merck) Germany, pump model L-7110, connected with a detector model L-7420.

The injector was a manual Rheodyne injector (Model 7161, Catati, California, USA) equipped with a 10\(\mu\)L\(^{1}\) injector loop and a 100\(\mu\)L Hamilton syringe. The instrument was connected to an IBM compatible PC bundled with Merck- Hitachi Model D-7000 chromatography Data Station software HPLC septum manager and an HP 800 inkjet printer.

A Lichrocart RP-18 column (250mm\(\times\)4.6mm i.d.) particle size 5 \(\mu\)m (Merck, Germany) was used for the analysis.

Procedures

Degradation of tadalafil.\(^{1}\)

Accurately weighed amount of 25 mg tadalafil then 10-mL of (0.8 N) hydrochloric acid was added, refluxed for 3 hin 100-mL of acetonitrile water mixture (1:1). During reflux, small portions were cooled and spotted on a TLC plate. Spotting was repeated at 30 min intervals to follow up time required for complete degradation. Solution was cooled, spotted and then developed using chloroform: acetone: ammonia (9:1:0.1, by volume) as a developing system.

Spectrophotometric methods

Spectral characteristics of tadalafil and its degradation product

Aliquot portions equivalent to (17.5\(\mu\)g \(\text{mL}^{-1}\)) tadalafil and its degrade were transferred into two 10-mL volumetric flasks from their stock solutions (250\(\mu\)g\(\text{mL}^{-1}\)) and completed to volume with acetonitrile water mixture (1:1). The zero order and the first derivative spectra of the prepared solutions were recorded.

Fourth derivative method

Accurately aliquots ranging from 10 to 70 \(\mu\)L equivalents to 2.5–17.5\(\mu\)g\(\text{mL}^{-1}\) at 10 \(\mu\)L intervals of tadalafil stock solution (250mg mL\(^{-1}\)) were transferred into 10-mL volumetric flasks then complete to volume with acetonitrile water mixture (1:1). The peak amplitudes of the obtained fourth derivative spectra were measured at 285.7 nm (\(\Delta\lambda = 4\text{nm}\)). A calibration curve relating the peak amplitude of the fourth derivative curve
at 285.7nm to the corresponding concentrations of tadalafil was constructed.

**Derivative ratio method**

Different aliquots of tadalafil stock solution (250µg/mL) in acetonitrile water mixture (1:1) ranging from 10 to 80 µL equivalents to 2.5 to 20 µg/mL at 10 µL intervals and 1.0 mL of the tadalafil degrade stock solution (100µg/mL) in acetonitrile water mixture (1:1) were transferred into 100-mL volumetric flasks then completed to volume with acetonitrile water mixture (1:1). The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer. The stored spectra of tadalafil were divided (amplitude at each wavelength) by the spectrum of 10 µg mL\(^{-1}\) standard tadalafil degrade.

The first derivative of the ratio spectra (1'_DD) were obtained at \(\Delta \lambda = 4\) nm. The amplitude of the first derivative peak of (tadalafil and its degrade) was measured at 263.6 nm and used to calculate the content of tadalafil. Calibration graph was constructed relating the peak amplitude of 1'_DD to the corresponding concentrations of tadalafil.

**Densitometric method**

Aliquot portions 2.5 – 40µL equivalents to 0.62 to 10 µgspot at 2.5 µL intervals of tadalafil stock solution (250µg/mL) in acetonitrile water mixture (1:1) was spotted on HPTLC plates using a Camag Applicator. Plates were developed by solvent system consisting of chloroform: acetone: ammonia (9:1:0.1, by volume) at room temperature. Plates were left to dry then the spots
were detected under UV-lamp (291 nm). A calibration curve relating the area under the peak to the corresponding concentrations of tadalafil was constructed over a range of 0.62 to 10 μgspot⁻¹

**Liquid chromatographic method**

**Linearity**

Aliquot portions 2.5 – 22.5 μg mL⁻¹ at 2.5mL intervals of tadalafil stock solution (250μg mL⁻¹) were transferred into a series of 10-mL volumetric flasks. The flasks were completed to volume with acetonitrile:water mixture (1:1). Aliquots (10μL) of the prepared solutions were injected into HPLC apparatus. The peak area was measured at 225 nm using a mobile phase consisting of water: acetonitrile: methanol in the ratio of (45:35:20) and a Lichrocart RP-18 (250mm×4.6mm
i.d.) column, particle size (5 µm) at a flow rate 1.2 mL min\(^{-1}\). A calibration graph representing the relative peak area of tadalafil to that of tadalafil external standard (10 µg mL\(^{-1}\)), versus the corresponding concentrations of tadalafil was constructed.

**Analysis of laboratory prepared mixtures containing different ratios from tadalafil and its degradation product**

**Fourth derivative and derivative ratio method**

Accurately aliquot portions equivalent to (2.5 – 17.50 µg mL\(^{-1}\)) of tadalafil and its degradation product (17.50 – 2.5 µg mL\(^{-1}\)) from their corresponding stock solutions (250 µg mL\(^{-1}\) in acetonitrile water mixture (1:1) were transferred into a series of 10-mL volumetric flasks as shown in Table 1. The volumes were completed with acetonitrile water mixture (1:1). Tadalafil concentration was calculated as mentioned under (Section 5.2.2) starting from (The peak amplitudes of the obtained fourth derivative…………) for the fourth derivative and (Section 5.2.3) starting from (The spectra of the prepared standard solutions…..) for the derivative ratio method.

**Densitometric method**

Aliquot portions equivalent to (0.62 – 10 µg spot\(^{-1}\)) of tadalafil and its degradation product (10 – 0.62 µg mL\(^{-1}\)) from their corresponding stock solutions (250 µg mL\(^{-1}\) in acetonitrile water mixture (1:1) were transferred into a series of 10-mL volumetric flasks as shown in Table 1. Aliquot portions 2.5 µL of the prepared mixtures were spotted on a HPTLC plate and developed as mentioned under (Section 5.3.1) starting from (Plates were developed by…………).

**Liquid chromatographic method**

Aliquot portions equivalent to (2.5 – 22.5 µg mL\(^{-1}\)) of tadalafil and its degradation product (22.5 – 2.5 µg mL\(^{-1}\)) from their stock solutions (250 µg mL\(^{-1}\) in acetonitrile water mixture (1:1) were transferred into a series of 10-mL volumetric flasks as shown in Table 1. Aliquot portions 25 µL of the prepared mixtures were injected into HPLC adopting the conditions under (Section 5.4.1) starting from (The peak area was measured……………).

**Analysis of tadalafil in pharmaceutical formulation**

The powder of 10 tablets, after unpacking, was weighed. An amount of the powder equivalent to 25 mg tadalafil was weighed into a 100-mL volumetric flask. A volume of 50 mL acetonitrile water mixture (1:1) was added. Mixture was stirred for 10 min using a vortex then filtered through a 0.45 µL membrane filter into a 100-mL volumetric flask and completed to volume with acetonitrile water mixture (1:1).

**RESULTS AND DISCUSSION**

**Degradation of tadalafil**

Tadalafil is a stable drug, however, forced stability
study under stress conditions revealed the instability of the drug in presence of hydrogen peroxide (Figure 2).

Tadalafil is soluble in acetonitrile water mixture (1:1). The molecular weight of tadalafil degragate was confirmed by mass spectroscopy. It was noted that 3 hours reflux using 0.8N hydrochloric acid was enough for complete degradation of tadalafil (Figure 3); this was demonstrated by the use of thin layer chromatography. Furthermore, complete shift of tadalafil UV-spectrum in acetonitrile water mixture (1:1) takes place (Figure 1).

**Derivative and derivative ratio spectrophotometric method**

**Derivative spectrophotometric method**

The zero, first, second and third order absorption spectra of tadalafil and its degradation products (Figure 4, 5, 6 and 7) respectively showed severe overlap that prevented the use of direct and derivative spectrophotometry for the analysis of tadalafil in presence of its degradation products.

Figure (4): Absorption spectrum of tadalafil 10µg mL⁻¹ (—) and its degradation product 10µg mL⁻¹ (---) using (acetonitrile : water) (1: 1v/v) as a solvent.

But fourth derivative applied to resolve such a mixture and to determine the concentration of tadalafil, (Fig-
The linearity was checked between the peak amplitude at the selected wavelength (285.7 nm) and the corresponding concentrations of tadalafil. A linear response was obtained for concentration range from 2.5 – 17.5 µg mL⁻¹. Figure 9.

The regression equation was found to be:

\[ {\text{D}}_{285.7} = 0.0025X + 0.0247 \]
\[ r = 0.9997 \]

Where \( X \) is the concentration of tadalafil in µg mL⁻¹, \( {\text{D}}_{285.7} \) is the amplitude of fourth derivative curve (tadalafil and its degradation) at 285.7 nm and \( r \) is the correlation coefficient.

**Derivative ratio spectrophotometric method**

The derivative ratio spectroscopy could be applied to resolve such a mixture and to determine the concentration of tadalafil. The zero order of the derivative ratio spectra of tadalafil, (Figure 10), and the first order of the derivative ratio spectra are presented in (Figure 11). The concentration of the divisor was also studied.

It was found that upon dividing by the spectrum of 2.5 µg mL⁻¹ degradation products give the best results in terms of sensitivity, repeatability and signals to noise ratio. The linearity was checked between the peak amplitude at the selected wavelength (263.6 nm) and the corresponding concentrations of tadalafil. Figure 12.

A linear response was obtained for concentration range from 2.5–20 µg mL⁻¹.

The regression equation was found to be:

\[ {\text{DD}}_{263.6} = 0.00445X - 0.0184 \]
\[ r = 0.9994 \]

Where \( X \) is the concentration of tadalafil in µg mL⁻¹, \( {\text{DD}}_{263.6} \) is the amplitude of first derivative curve (tadalafil and its degradation) at 263.6 nm and \( r \) is the correlation coefficient.
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Densitometric method
A densitometric method is described for the determination of tadalafil in presence of its degradation product without prior separation. Different solvent systems were tried for the separation of tadalafil from its degradation product. Satisfactory results were obtained by using chloroform: ammonia (9:1:0.1, by volume) as a developing system ($R_f = 0.41, 0.62$ for tadalafil and degrade, respectively). The separation allows the determination of tadalafil with no interference from its degrade (Figure. 13).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentration at 291 nm over a range of $0.62 \text{–} 10 \mu g \text{ mL}^{-1}$ where a linear response was obtained (Figure 14).

The regression equation was found to be:

$$A = 1162.1X + 892.49, \ r = 0.9996$$

Where $A$ is the integrated area under the peak for tadalafil, $X$ is the concentration in $\mu g \text{ spot}^{-1}$ for tadalafil and $r$ is the regression coefficient.

Liquid chromatographic method
A simple isocratic high-performance liquid chromatographic method is described for the determination of tadalafil in presence of its degradation product. System suitability parameters were tested by calculating the capacity factor, tailing factor, the selectivity factor and resolution. Best peak shape was obtained water: acetonitrile: methanol in the ratio of (45:35:20) with resolution times of $4.7 \pm 0.03$ and $2.18 \pm 0.03$ min for tadalafil and its degrade, respectively (Figure. 15).

A linear response was obtained between the relative peak area and the corresponding concentrations of the tadalafil in the range of $2.5 \text{–} 25 \mu g \text{ mL}^{-1}$. The regression equation was found to be: (Figure. 16).

$$A = 9.5542X + 0.0082, \ r = 0.9998$$

Where $A$ is the relative area under the peak, $X$ is the concentration in $\mu g \text{ mL}^{-1}$ and $r$ is the regression coefficient.

Method validation
The selectivity and specificity of the proposed method...
Figure 13: Two dimensional TLC-Separation of tadalafil from its degradate

\[ y = 1162.1x + 892.49 \]
\[ r = 0.9996 \]

Figure 14: Linearity of the integrated peak area to the corresponding concentration of tadalafil (\(\mu g \text{ spot}^{-1}\))

Column: RP 18
Injection volume: 10.00 \(\mu L\).
Flow rate: 1.20 \(mL \text{ min}^{-1}\).
Detection: 225 nm.

Figure 15: HPLC chromatogram of tadalafil (10 \(\mu g \text{ mL}^{-1}\), \(R_t: 4.7 \text{ min}\)) its degradation product (10 \(\mu g \text{ mL}^{-1}\), \(R_t: 2.18 \text{ min}\)) using the specified chromatographic conditions
Stability-indicating methods for determination of tadalafil in presence of its degradation product

Figure 16: Linearity of the peak area ratio to the corresponding concentration of tadalafil

TABLE 1: Determination of tadalafil in laboratory prepared mixtures by the proposed derivative, derivative ratio, densitometry and HPLC method

<table>
<thead>
<tr>
<th>Drug Conc. (µg mL⁻¹)</th>
<th>% of Degradation</th>
<th>⁴ᵗʰ Derivative</th>
<th>¹ˢᵗ Derivative ratio</th>
<th>HPLC Conc. (µg spot⁻¹)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>90 %</td>
<td>99.10</td>
<td>99.57</td>
<td>101.90</td>
<td>1.00</td>
</tr>
<tr>
<td>5.00</td>
<td>80 %</td>
<td>101.06</td>
<td>100.65</td>
<td>101.30</td>
<td>2.00</td>
</tr>
<tr>
<td>7.50</td>
<td>70 %</td>
<td>101.4</td>
<td>99.88</td>
<td>100.76</td>
<td>3.00</td>
</tr>
<tr>
<td>10.00</td>
<td>60 %</td>
<td>100.52</td>
<td>101.59</td>
<td>99.97</td>
<td>4.00</td>
</tr>
<tr>
<td>12.50</td>
<td>50 %</td>
<td>99.30</td>
<td>101.97</td>
<td>101.75</td>
<td>5.00</td>
</tr>
<tr>
<td>15.00</td>
<td>40 %</td>
<td>100.34</td>
<td>99.26</td>
<td>100.21</td>
<td>6.00</td>
</tr>
<tr>
<td>17.50</td>
<td>30 %</td>
<td>99.52</td>
<td>7.00</td>
<td>99.19</td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td>20 %</td>
<td>99.49</td>
<td>8.00</td>
<td>101.93</td>
<td></td>
</tr>
<tr>
<td>22.50</td>
<td>10 %</td>
<td>100.84</td>
<td>100.63</td>
<td>100.53</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>100.45</td>
<td>100.39</td>
<td>100.63</td>
<td>100.53</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>1.130</td>
<td>0.904</td>
<td>0.902</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2: Determination of tadalafil in Cialis® tablets by the proposed procedures

<table>
<thead>
<tr>
<th>Cialis tablet</th>
<th>⁴ᵗʰ Derivativemethod Recovery % ±S.D. (a)</th>
<th>¹ˢᵗ Derivative ratio method Recovery % ±S.D. (a)</th>
<th>Densitometric method</th>
<th>HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage form BN: A779729</td>
<td>100.89 ± 0.888</td>
<td>100.57 ± 1.005</td>
<td>100.59 ± 0.749</td>
<td>100.46 ± 0.910</td>
</tr>
</tbody>
</table>

(a) Average of seven determinations

Methods were verified by determination of tadalafil in laboratory prepared mixture containing different ratios of the drug and its degradation product. The analysis was valid up to 90% of the degradation product for the (⁺D), (⁻DD) method, densitometry and liquid chromatography, respectively (TABLE 1).

To ascertain the accuracy of the proposed procedures, they were successfully applied for the determination of tadalafil in Cialis® tablets as presented in TABLE 2.

The validity of the proposed procedures was further assessed by application of the standard addition
The small relative standard deviations indicate that the methods were accurate (TABLE 3).

The precision of the suggested methods was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis. The individual methods were also checked for its robustness by minor changes in assay conditions, the methods proved robust. The obtained assay parameters and a validation sheet are presented in (TABLE 4).

The results obtained by the proposed methods for

### TABLE 3: Results of application of standard addition to the determination of tadalafil in Cialis® tablets by the proposed methods

<table>
<thead>
<tr>
<th>Cialis tablet</th>
<th>Claimed taken (µg mL⁻¹)</th>
<th>Pure added (µg mL⁻¹)</th>
<th>4ᵗʰ Derivative</th>
<th>1ˢᵗ Derivative ratio</th>
<th>Densitometry</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage form BN: A779729</td>
<td>2.50</td>
<td>100.44</td>
<td>100.43</td>
<td>101.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>99.74</td>
<td>100.37</td>
<td>101.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.50</td>
<td>99.80</td>
<td>101.19</td>
<td>100.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.00</td>
<td>101.60</td>
<td>101.28</td>
<td>100.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.50</td>
<td>100.43</td>
<td>99.97</td>
<td>100.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.00</td>
<td>101.04</td>
<td>100.31</td>
<td>99.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.50</td>
<td>(7.50)</td>
<td>101.06</td>
<td>101.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td></td>
<td>99.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.50</td>
<td></td>
<td></td>
<td>100.63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.D. | 100.53 ± 0.715 | 100.46 ± 0.669 | 100.56 ± 0.634 | 100.51 ± 0.894 |

### TABLE 4: Assay parameters and validation sheet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4ᵗʰ Derivative method</th>
<th>1ˢᵗ Derivative ratio method</th>
<th>Densitometric method</th>
<th>HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Range (µg mL⁻¹)</td>
<td>2.50–17.50</td>
<td>7.50–20.00</td>
<td>0.62–10.00</td>
<td>2.50–25.00</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0025</td>
<td>0.0445</td>
<td>1162.1</td>
<td>9.5542</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0247</td>
<td>-0.0184</td>
<td>892.49</td>
<td>0.0082</td>
</tr>
<tr>
<td>Mean</td>
<td>100.52</td>
<td>100.59</td>
<td>100.45</td>
<td>100.47</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.045</td>
<td>0.89</td>
<td>0.726</td>
<td>0.676</td>
</tr>
<tr>
<td>Variance</td>
<td>1.022</td>
<td>0.943</td>
<td>0.852</td>
<td>0.822</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>1.039</td>
<td>0.884</td>
<td>0.722</td>
<td>0.672</td>
</tr>
<tr>
<td>Correlation Coefficient (r)</td>
<td>0.9997</td>
<td>0.9997</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.025</td>
<td>0.829</td>
<td>0.726</td>
<td>0.725</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.029</td>
<td>0.836</td>
<td>0.732</td>
<td>0.728</td>
</tr>
</tbody>
</table>

### TABLE 5: Statistical comparison for the results obtained by the proposed methods and the compendia method for the analysis of tadalafil in bulk powder

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4ᵗʰ Derivative method</th>
<th>1ˢᵗ Derivative ratio method</th>
<th>Densitometry</th>
<th>HPLC</th>
<th>Compendia method (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>100.52</td>
<td>100.57</td>
<td>100.52</td>
<td>100.47</td>
<td>101.40</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.045</td>
<td>0.827</td>
<td>0.726</td>
<td>0.717</td>
<td>1.64</td>
</tr>
<tr>
<td>Variance</td>
<td>1.022</td>
<td>0.683</td>
<td>0.527</td>
<td>0.514</td>
<td>1.28</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>F-test</td>
<td>1.252 (6.16)*</td>
<td>1.357 (6.16)*</td>
<td>1.502 (6.09)*</td>
<td>1.577 (6.00)*</td>
<td></td>
</tr>
<tr>
<td>Student’s t-test</td>
<td>1.417 (2.228)*</td>
<td>1.332 (2.228)*</td>
<td>1.539 (2.201)*</td>
<td>1.730 (2.160)*</td>
<td></td>
</tr>
</tbody>
</table>

*The figures in parenthesis are the corresponding tabulated values at P = 0.05 (Remington)
determination of tadalafil in bulk powder were statistically compared with those obtained by applying the compendial method\[14\], and it revealed insignificant difference (TABLE 5).

**ABBREVIATION**

1D - First derivative.
2D - Second derivative.
3D - Third derivative.
4D - Fourth derivative.
1DD - First derivative ratio.
HPLC - High performance liquid chromatography.
HPTLC - High performance thin layer chromatography.
TD - Tadalafil.
TLC - Thin layer chromatography.

**CONCLUSION**

The proposed procedures are simple, sensitive, selective and stability indicating. The methods can be used for the routine analysis of tadalafil either in bulk powder or in pharmaceutical dosage forms. The proposed methods can be applied in laboratories lacking sophisticated instruments such as GC–MS or LC–MS. The suggested methods can be simply applied to kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceuticals.

**REFERENCES**