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

Moataz A. Yehia*, Mamdouh R. Rezk, Mohamed A. El-Sayed, Mohamed Abdel Kawy
Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, ET 11562, Cairo, (EGYPT)
E-mail: mayehialiaa2010@hotmail.com; boodim3@yahoo.com

INTRODUCTION

Sildenafil (SD), is 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4, 3-d] pyrimidin-5-yl) phenylsulfonyl]-4-methylpiperazine. (Figure 1) It is phosphodiesterase-5 inhibitors used for erectile dysfunction. Sildenafil is 80% eliminated mainly in the faeces, 13% eliminated in urine. The literature has analytical methods for quantitative estimation of sildenafil, in pharmaceutical formulations by spectroscopic methods[3-7], Densitometric[8], GC[9-14], HPLC[15-29], and capillary zone electrophoresis[30,31].

The present work aimed to develop feasible, sensitive and specific analytical procedures for the analysis of sildenafil 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
- Sildenafil powder was kindly supplied by Pfizer PGM Company, France.
- Pharmaceutical formulation Viagra® tablets (la-
beled to contain 50 mg of sildenafil), manufactured by Pfizer PGM Company.

Reagents

The materials used were Chloroform, acetic acid, ethanol, hydrogen peroxide, methanol, toluene, ADWIC, El Nasr Pharmaceutical Co. (Cairo, Egypt); Purified water was prepared using a Millipore Milli-Q water purification system, all were of analytical grade. Acetonitrile, methanol: Merck (Darmstadt, Germany), all were of HPLC grade, all Chemical were purchased from local market (Cairo, Egypt).

Standard solutions

Sildenafil standard and degradate solution (100 µg mL⁻¹) in methanol.

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 F₂₅₄ (20 cm x 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 µL) (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 µL⁻¹ injector loop and a 100 µL 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 × 4.6mm i.d.) particle size 5 µm (Merck, Germany) was used for the analysis.

Procedures

Degradation of sildenafil

Into a round bottom conical flask weighed, an amount of 100 mg sildenafil citrate was dissolved in 100-mL of methanol transferred 2-mL of 30% hydrogen peroxide were added, (portions of methanol were added periodically to maintain the volume). After 6 hours reflux, complete degradation was achieved, as tested by TLC using chloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volumes) as a developing solvent. Solvent was evaporated and solid powder was characterized.

Spectrophotometric methods

Spectral characteristics of sildenafil and its degradation product

Two portion of sildenafil and its degradate were separately, transferred into two 10-mL volumetric flasks from their secondary stock solutions (100 µg mL⁻¹) and the volume was completed with methanol to obtain solutions having the concentration (24 µg mL⁻¹).

The zero, first, second, third and fourth order
spectra of the prepared solutions were recorded and investigated.

**Fourth derivative method**

Different aliquots (0.2 – 3 mL) were taken from sildenafil secondary stock solution (100 µg mL\(^{-1}\)) into 10-mL volumetric flasks then the volume was completed with methanol, to get a concentration range (2 – 30 µg mL\(^{-1}\)) TABLE I. The fourth derivative of the standard spectra for sildenafil and its degradation product were obtained using (Δλ= 4 nm) and scaling factor 100, the peak amplitudes at 292.4 nm were recorded, and plotted against the corresponding concentration. The regression equation was obtained that was used for determination of sildenafil in unknown sample.

**Derivative ratio method**

The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer. The stored spectra of sildenafil were divided (amplitude at each wavelength) by the spectrum of 10 µg mL\(^{-1}\)sildenafil degradate.

The first derivative of the ratio spectra (\(1\text{DD}\)) were obtained at Δλ= 4 nm and scaling factor was 10. The amplitude of the first derivative peak of sildenafil was measured at 305.4 nm and used to calculate the concentration of sildenafil. Calibration graph was constructed relating the peak amplitude of \(1\text{DD}_\text{305.4}\) to the corresponding concentrations of sildenafil citrate.

**Densitometric method**

Aliquot portions (5 – 80 µL) equivalents to 0.5 to 8 µg spot\(^{-1}\)TABLE I of sildenafil stock solution (100 µg mL\(^{-1}\)) in methanol was spotted on HPTLC plates using a Camag Applicator. Plates were developed by solvent system consisting of chloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volume) at room temperature. Plates were left to dry then the spots were detected under UV-lamp (292 nm). A calibration curve relating the area under the peak to the corresponding concentrations of sildenafil was constructed over a range of 0.5 to 8 µg spot\(^{-1}\)

**Liquid chromatographic method**

**Linearity**

Aliquot portions (0.5 – 8 mL) of sildenafil stock solution (100 µg mL\(^{-1}\)) were transferred into a series of 10-mL volumetric flasks. The flasks were completed to volume with methanol in concentration range 5 to 80 µg mL\(^{-1}\)TABLE I. Aliquots (10 µL)

---

### TABLE 1: Determination of sildenafil in pure samples by derivative and derivative ratio methods

<table>
<thead>
<tr>
<th></th>
<th>Fourth derivative spectroscopic method (µg mL(^{-1}) at 292.4 nm)</th>
<th>Derivative ratio spectroscopic method (µg mL(^{-1}) at 305.4 nm)</th>
<th>Densitometry (µg spot(^{-1}))</th>
<th>HPLC (µg mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found</td>
<td>Found %</td>
<td>Taken</td>
<td>Found</td>
</tr>
<tr>
<td>2.00</td>
<td>2.01</td>
<td>100.50</td>
<td>2.50</td>
<td>2.51</td>
</tr>
<tr>
<td>4.00</td>
<td>4.01</td>
<td>100.25</td>
<td>5.00</td>
<td>5.06</td>
</tr>
<tr>
<td>8.00</td>
<td>8.08</td>
<td>101.00</td>
<td>7.50</td>
<td>7.48</td>
</tr>
<tr>
<td>10.00</td>
<td>9.91</td>
<td>99.10</td>
<td>10.00</td>
<td>10.04</td>
</tr>
<tr>
<td>12.00</td>
<td>11.95</td>
<td>99.58</td>
<td>12.50</td>
<td>12.67</td>
</tr>
<tr>
<td>16.00</td>
<td>16.18</td>
<td>101.12</td>
<td>15.00</td>
<td>14.89</td>
</tr>
<tr>
<td>18.00</td>
<td>17.98</td>
<td>99.88</td>
<td>17.50</td>
<td>17.77</td>
</tr>
<tr>
<td>20.00</td>
<td>20.08</td>
<td>100.40</td>
<td>20.00</td>
<td>19.84</td>
</tr>
<tr>
<td>24.00</td>
<td>24.08</td>
<td>101.31</td>
<td>22.50</td>
<td>22.56</td>
</tr>
<tr>
<td>26.00</td>
<td>26.07</td>
<td>100.26</td>
<td>25.00</td>
<td>25.48</td>
</tr>
<tr>
<td>28.00</td>
<td>27.82</td>
<td>99.35</td>
<td>27.50</td>
<td>27.41</td>
</tr>
<tr>
<td>30.00</td>
<td>30.25</td>
<td>100.83</td>
<td>30.00</td>
<td>29.77</td>
</tr>
<tr>
<td>Mean</td>
<td>100.29</td>
<td>100.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.710</td>
<td>0.965</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD%</td>
<td>0.708</td>
<td>0.961</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of the prepared solutions were injected into HPLC apparatus. The peak area was measured at 225 nm using a mobile phase consisting of (acetonitrile: methanol: 0.05 M potassium di-hydrogen phosphate (v/v, pH 5.8): tri-ethyl amine, in the ratio of (45: 25: 30: 0.2, by volumes) and a Lichrocart RP-18 (250mm×4.6mm i.d.) column, particle size (5µm) at a flow rate 1.0 mL min\(^{-1}\). A calibration graph representing the relative peak area of sildenafil to that of sildenafil external standard (20µg mL\(^{-1}\)), versus the corresponding concentrations of sildenafil was constructed.

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

**Fourth derivative and derivative ratio method**

Accurately aliquot portions (0.2 – 1.8 mL), (0.4 – 1.6 mL) for derivative and derivative ratio respectively, of sildenafil and sildenafil degrade from their corresponding stock solutions (100 µg mL\(^{-1}\) in methanol) were transferred into a series of 10 mL volumetric flasks as shown in TABLE 1. The volumes were completed with methanol. Sildenafil 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 (8 – 72µL) of sildenafil and degrade from their corresponding stock solutions (100µg mL\(^{-1}\) in methanol 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 (0.5 – 6.5 mL) of sildenafil and degrade from their corresponding stock solutions (100µg mL\(^{-1}\) in methanol were transferred into a series of 10 mL volumetric flasks as shown in TABLE 1. Aliquot portions 10 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 sildenafil in pharmaceutical formulation**

Ten tablets were weighed to determine the average weight per tablet then grinded. A mass of
Viagra® powder tablets equivalent to 10 mg sildenafil was transferred into a 100-mL volumetric flask. A volume of 50-mL methanol was added. Stirring was done for 10 minutes using a vortex then filtration was done and the procedure under linearity with secondary stock solution (100 µg mL⁻¹) and concentration range (2.5 – 30 µg mL⁻¹), was done for the derivative ratio method. The concentration of sildenafil
was estimated from the regression equation.

RESULTS AND DISCUSSION

Degradation of sildenafil

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

Sildenafil is soluble in methanol. The molecular weight of sildenafil degradeate was confirmed by mass spectroscopy. It was noted that 6 hours reflux using 30% hydrogen peroxide was enough for complete degradation of sildenafil (Figure 2); this was demonstrated by the use of thin layer chromatography. Furthermore, complete shift of sildenafil UV-spectrum in methanol takes place (Figure 1).

Derivative and derivative ratio spectrophotometric method

Derivative spectrophotometric method

The zero, first, second and third order absorption spectra of sildenafil and its degradation products (Figure 3, 4, 5 and 6) respectively showed severe overlap that prevented the use of direct and derivative spectrophotometry for the analysis of sildenafil in presence of its degradation products.

But fourth derivative applied to resolve such a mixture and to determine the concentration of sildenafil, (Figure 7)

The linearity was checked between the peak

![Figure 5: Second derivative spectra of sildenafil and its degradation product 24 µg mL⁻¹ using methanol as a solvent](image)

![Figure 6: Third derivative spectra of sildenafil and its degradation product 24 µg mL⁻¹ using methanol as a solvent](image)
Figure 7: Fourth derivative spectra of sildenafil (—) and its degradation product 24 µg mL\(^{-1}\) (---) using methanol as a solvent.

Figure 8: Linearity of the peak amplitude of the forth derivative curve at 292.4 nm to the corresponding concentration of sildenafil.

Figure 9: Zero order of ratio spectra of sildenafil 2.5 – 30 µg mL\(^{-1}\) of its degradation product as a divisor using methanol as a solvent.
Figure 10: First order of derivative ratio spectra of sildenafil 2.5 – 30 µg mL\(^{-1}\) using 10µg mL\(^{-1}\) of its degradation product as a divisor using methanol as a solvent.

Figure 11: Linearity of the peak amplitude of the first derivative of the ratio spectra at 305.4 nm against concentration of sildenafil.

Figure 12: Two dimensional TLC-Separation of sildenafil from its degradation amplitude at the selected wavelength (292.4 nm for concentration range from 2 – 30 µg mL\(^{-1}\). Figure (8).

The regression equation was found to be:

\[ 4D_{292.4} = 0.015X + 0.0505r = 0.9997 \]

Where X is the concentration of sildenafil in µg mL\(^{-1}\), \(4D_{292.4}\) is the amplitude of fourth derivative curve (sildenafil and its degradation) at 292.4 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 sildenafil. The zero order of the derivative ratio spectra of sildenafil, (Figure 9), and the first order of the derivative ratio spectra are presented in (Figure 10). The concentration of the divisor was also studied.

It was found that upon dividing by the spectrum of 10 µg mL⁻¹ degradation product gave 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 (305.4 nm) and the corresponding concentrations of sildenafil. Figure (11).

A linear response was obtained for concentration range from 2.5–30 µg mL⁻¹.

The regression equation was found to be:

\[ 1DD_{305.4} = 0.0441X - 0.0003 \]

Where \( X \) is the concentration of sildenafil in µg mL⁻¹, \( 1DD_{305.4} \) is the amplitude of first derivative curve (sildenafil and its degrade) at 305.4 nm and \( r \) is the correlation coefficient.

Densitometric method

A densitometric method is described for the determination of sildenafil presence of its degrade without prior separation. Different solvent systems were tried for the separation of sildenafil from its degradation product. Satisfactory results were obtained by using chloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volume) as a developing system.
(R<sub>f</sub> = 0.45, 0.08 for sildenafil and degradate, respectively). The separation allows the determination of sildenafil with no interference from its degradate (Figure 12).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentration at 292 nm over a range of 0.5 – 8 µg spot<sup>-1</sup> where a linear response was obtained. Figure (13).

The regression equation was found to be:

\[ A = 0.6741X + 0.1148, \quad r = 1 \]

Where A is the integrated area under the peak (x 10<sup>-3</sup>), for sildenafil, X is the concentration in µg spot<sup>-1</sup>

![Figure 15](image)

**Figure 15**: Linearity of the peak area ratio to the corresponding concentration of sildenafil

<table>
<thead>
<tr>
<th>Recovery for sildenafil by (%)</th>
<th>Spectroscopy(µg mL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>HPLC (µg mL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Densitometry (µg spot&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>Fourth Derivative Recovery %</td>
<td>Derivative ratio Recovery %</td>
<td>Conc.</td>
</tr>
<tr>
<td>2.00</td>
<td>100.79</td>
<td>101.28</td>
<td>5</td>
</tr>
<tr>
<td>4.00</td>
<td>100.56</td>
<td>101.86</td>
<td>10</td>
</tr>
<tr>
<td>6.00</td>
<td>98.18</td>
<td>101.86</td>
<td>20</td>
</tr>
<tr>
<td>8.00</td>
<td>99.37</td>
<td>99.56</td>
<td>30</td>
</tr>
<tr>
<td>10.00</td>
<td>101.4</td>
<td>99.14</td>
<td>40</td>
</tr>
<tr>
<td>12.00</td>
<td>99.03</td>
<td>100.1</td>
<td>50</td>
</tr>
<tr>
<td>14.00</td>
<td>100.14</td>
<td>100.21</td>
<td>60</td>
</tr>
<tr>
<td>16.00</td>
<td>102.08</td>
<td>101.09</td>
<td>65</td>
</tr>
<tr>
<td>18.00</td>
<td>99.34</td>
<td></td>
<td>7.20</td>
</tr>
<tr>
<td>Mean</td>
<td>100.09</td>
<td>100.46</td>
<td>99.71</td>
</tr>
<tr>
<td>S.D</td>
<td>1.237</td>
<td>0.980</td>
<td>1.059</td>
</tr>
</tbody>
</table>
for sildenafil and $r$ is the regression coefficient.

**Liquid chromatographic method**

A simple isocratic high-performance liquid chromatographic method is described for the determination of sildenafil 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 acetonitrile: methanol: 0.05 M potassium di-hydrogen phosphate,(pH 5.8): tri-ethyl amine (45: 25: 30: 0.2, by volume) with retention times of 8.83 ± 0.02 minutes and 6.23 ± 0.02 minutes for sildenafil and its degradate, respectively (Figure 14).

A linear response was obtained between the relative peak area and the corresponding concentrations of the sildenafil in the range of 5 – 80 $\mu$g mL$^{-1}$. The regression equation was found to be: (Figure 15).

$$A = 0.055X - 0.0939$$

$A$ = 0.055$X$ - 0.0939

$A$ = 0.055$X$ - 0.0939

$r = 0.9998$

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

**Method validation**

**TABLE 3 : Determination of sildenafil in Viagra® tablets by the proposed procedures**

<table>
<thead>
<tr>
<th>Dosage form BN: 9197205</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>100.22 ± 0.809</td>
<td>100.22 ± 0.680</td>
<td>100.48 ± 0.767</td>
<td>100.77 ± 0.693</td>
<td></td>
</tr>
</tbody>
</table>

(a) Average of seven determinations

**TABLE 4 : Results of application of standard addition to the determination of sildenafil in Viagra® tablets by the proposed methods**

<table>
<thead>
<tr>
<th>Viagra® tablet</th>
<th>Fourth Derivative (Claimed taken: Standard added) (Recovery %) ( $\mu$g mL$^{-1}$)</th>
<th>Derivative ratio (Claimed taken: Standard added) (Recovery %) ( $\mu$g mL$^{-1}$)</th>
<th>Densitometry (Claimed taken: Standard added) (Recovery %) (ugspot$^{-1}$)</th>
<th>HPLC (Claimed taken: Standard added) (Recovery %) ( $\mu$g mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2.00: 2.00) 100.19 (2.50: 2.50) 101.82</td>
<td>(2.50: 2.50) 101.82</td>
<td>(0.50: 0.50) 101.08</td>
<td>(5.00: 5.00) 99.38</td>
<td></td>
</tr>
<tr>
<td>(2.00: 4.00) 102.10 (2.50: 5.00) 101.67</td>
<td>(2.50: 5.00) 101.67</td>
<td>(0.50: 1.00) 101.04</td>
<td>(5.00: 10.00) 101.09</td>
<td></td>
</tr>
<tr>
<td>(2.00: 6.00) 99.70 (2.50: 7.50) 99.54</td>
<td>(2.50: 7.50) 99.54</td>
<td>(0.50: 2.00) 100.48</td>
<td>(5.00: 20.00) 99.10</td>
<td></td>
</tr>
<tr>
<td>(2.00: 8.00) 99.17 (2.50: 10.00) 100.43</td>
<td>(2.50: 10.00) 100.43</td>
<td>(0.50: 3.00) 100.39</td>
<td>(5.00: 30.00) 101.39</td>
<td></td>
</tr>
<tr>
<td>(2.00: 10.00) 98.94 (2.50: 12.50) 100.77</td>
<td>(2.50: 12.50) 100.77</td>
<td>(0.50: 4.00) 100.86</td>
<td>(5.00: 40.00) 99.89</td>
<td></td>
</tr>
<tr>
<td>(2.00: 12.00) 100.15 (2.50: 15.00) 99.72</td>
<td>(2.50: 15.00) 99.72</td>
<td>(0.50: 5.00) 100.43</td>
<td>(5.00: 50.00) 101.10</td>
<td></td>
</tr>
<tr>
<td>(2.00: 14.00) 98.57 (2.50: 17.50) 100.40</td>
<td>(2.50: 17.50) 100.40</td>
<td>(0.50: 6.00) 100.73</td>
<td>(5.00: 60.00) 101.47</td>
<td></td>
</tr>
<tr>
<td>(2.00: 16.00) 99.79 (2.50: 20.00) 99.25</td>
<td>(2.50: 20.00) 99.25</td>
<td>(0.50: 7.00) 101.73</td>
<td>(5.00: 70.00) 101.36</td>
<td></td>
</tr>
<tr>
<td>(2.00: 18.00) 101.03 (2.50: 22.50) 100.97</td>
<td>(2.50: 22.50) 100.97</td>
<td>Mean ± S.D. 99.96 ± 1.091</td>
<td>100.61 ± 0.947</td>
<td>100.72 ± 0.508</td>
</tr>
</tbody>
</table>

By applying the proposed HPLC method, it was possible to determine sildenafil citrate in its pure powder form. The mean accuracy was found as shown in (TABLES 1).

The selectivity and specificity of the proposed methods were verified by determination of sildenafil 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 (4’D), (1’DD) method, densitometry and liquid chromatography, respectively (TABLES 2).

To ascertain the accuracy of the proposed procedures, they were successfully applied for the determination of sildenafil in Viagra® tablets as presented in TABLE 3.

The validity of the proposed procedures was further assessed by application of the standard addition technique. The small relative standard deviations indicate that the methods were accurate (TABLE 4).

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 5).

The results obtained by the proposed methods for determination of sildenafil in bulk powder were statistically compared with those obtained by applying the reported method\[20\], and it revealed insignificant difference (TABLE 6).

### CONCLUSION

The proposed procedures are simple, sensitive, selective and stability indicating. The methods can be used for the routine analysis of sildenafil 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.

### ABBREVIATION

1D.........First derivative.
2D........Second derivative.
3D........Third derivative.
4D..........Forth derivative.
1DD.........First derivative ratio.
HPLC......High performance liquid chromatography.
HPTLC...High performance thin layer chromatography.
SD.........Sildenafil.
TLC.......Thin layer chromatography.

### REFERENCES


