DEVELOPMENT AND VALIDATION OF A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TADALAFIL AND DAPOXETINE

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ABSTRACT:
A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Tadalafil and Dapoxetine in bulk and dosage forms. The method involves separation on Xterra C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1% OPA (pH 2.8) and Methanol (45:55v/v) with a flow rate of 1ml/min and UV detection at 229mn. Retention time was 2.78 min (Tadalafil), 3.71 min (Dapoxetine). Linearity range was 20-100 ug/ml (Tadalafil), 60-300 ug/ml (Dapoxetine). Accuracy was in the range of 96.92-100.35% for both drugs. Precision was 0.8% and 0.5% for Tadalafil and Dapoxetine. LOD and LOQ are 0.42ug/ml and 1.38 ug/ml for Tadalafil, 0.27ug/ml and 0.89ug/ml for Dapoxetine. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay,
drug content was within 98.55-101.4 % of labelled content. Forced degradation studies indicated the suitability of the method for stability studies.

KEY WORDS:
Tadalafil and Dapoxetine, RP-HPLC Method, Simultaneous estimation, Validation, Forced degradation studies.

INTRODUCTION:
Erectile dysfunction and premature ejaculation are the two most prevalent male sexual dysfunctions. They affect physical and psychosocial health and has a significant impact on the quality of life of sufferers and their partners. Combination therapy of Tadalafil and dapoxetine has been proven to be effective and safe for treatment of erectile dysfunction and premature ejaculation.

Tadalafil (TDL) belongs to category of phosphodiesterase type-5 inhibitor. It is recommended for the treatment of male erectile dysfunction. It inhibits cGMP specific PDE5 which is responsible for degradation of cGMP in the corpus cavernosum located around the penis.

Dapoxetine an oral selective serotonin reuptake inhibitor indicated for the treatment of premature ejaculation. It is mainly used as erectile dysfunction as selective short acting potent serotonin reuptake inhibitor (SSRI). Dapoxetine HCL mechanism of action is thought to be inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time.

Though several methods are reported in literature for the estimation of Tadalafil and Dapoxetine individually, there are only few HPLC methods reported for the simultaneous estimation of Tadalafil and Dapoxetine combination.

The objective of the present study was to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Tadalafil and Dapoxetine and validate the method with forced degradation studies according to ICH guidelines.
MATERIALS AND METHODS:

Materials and reagents:

HPLC water (Lichrosolv® Merck Lifesciences Pvt. Ltd., Mumbai, India) Ortho phosphoric acid (Thermo Fischer Scientific Pvt Ltd., Mumbai, India) were used in the study. The working standards of Tadalafil and Dapoxetine were generous gift obtained from Pharma Train Pvt Ltd., Hyderabad, India. Duraplus tablet containing Tadalafil 10mg and Dapoxetine 30mg was kindly supplied by Sun pharmaceutical Industries Ltd.

Instrumentation:

Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) with an autosampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV crossinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital Ph meter (Systronics model 802) were used in the study.

Chromatography conditions:

The chromatographic conditions was performed on XTerra C18 column (250 X 4.6mm, 5µm particle size) at an ambient column temperature. The samples were eluted using 0.1% Ortho phosphoric acid (pH adjusted to 2.8): Methanol (45:55v/v) as the mobile phase at a flow rate of 1 ml/min and samples were degassed by ultrasonication for 20 min and filtered through 0.45µm Nylon(N66)47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1.0 mL/min, and UV detection was carried out at 229nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Tadalafil and Dapoxetine were recorded under optimized chromatographic conditions (Fig. 1).

Diluent

Buffer and Methanol in 50:50 v/v ratio.

Preparation of Standard Solutions:

Stock standard solution:

Standard stock solution of Tadalafil and Dapoxetine were prepared by dissolving 20 mg of Tadalafil and 60mg of Dapoxetine in 10ml of diluent (Buffer: Methanol, 50:50v/v) in a 10ml clean dry volumetric flask and
the standard solutions was filtered through 0.45μm nylon membrane filter and degassed by sonicator to get the concentration of 2000µg/ml of Tadalafil and 6000µg/ml of Dapoxitine. The above standard stock solution suitably diluted with diluents to obtain working concentration drugs.

**Working Standard Solution:**

Working standard solution of Tadalafil and Dapoxitine were prepared by taking 0.3ml of stock solutions of Tadalafil and Dapoxitine in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 60µg/ml of Tadalafil and 180µg/ml of Dapoxitine.

**Preparation of Sample Solutions of Tadalafil and Dapoxitine:**

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 20 mg of Tadalafil and 60 mg of Dapoxitine was taken into 10 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution suitably diluted to get a concentration of 60µg/ml of Tadalafil and 180µg/ml of Dapoxitine.

**RESULTS AND DISCUSSION:**

**Optimization of chromatographic conditions:**

Tadalafil and Dapoxitine were soluble in polar solvent, so the developed method of estimation was carried out on reverse phase high performance liquid chromatography. To develop a rugged and suitable Preliminary trials were taken with different composition of buffer and organic phase of mobile phases with pH range of 2.5–5. After evaluating all these factors, a XTerra C18 column was found to be giving satisfactory results. The selection of methanol and buffer were based on chemical structure of both the drugs. Best results were obtained with 0.1% O-phosphoric acid pH adjusted to 2.8 with sodium hydroxide solution that improved the peak shapes of Tadalafil and Dapoxitine. Mobile phase composition consisting of a mixture of buffer-pH 2.8 (0.1% Ortho phosphoric acid): Methanol (45:55v/v). Flow rates between 0.5 to 1.2ml/min were tried. Flow rate of 1ml/min was observed to be enough to get both the drugs eluted within less than 10min. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

**Validation of Method Developed:**

The proposed method was validated according to the ICH guidelines for the following parameters
System suitability:

The Retention time of Tadalafil and Dapoxetine using optimum conditions was 2.78min and 3.72min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Tadalafil and Dapoxetine was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in Table 1.

Table 1: System suitability results of Tadalafil and Dapoxetine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tadalafil</th>
<th>Dapoxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td>188339 (0.46%)*</td>
<td>1092032 (0.14%)*</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>2675.81±0.302</td>
<td>3626.04±0.258</td>
</tr>
<tr>
<td>Retention time</td>
<td>2.789±0.031</td>
<td>3.726±0.057</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.10±0.07</td>
<td>1.27±0.05</td>
</tr>
</tbody>
</table>

Specificity:

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized chromatogram of Tadalafil and Dapoxetine is shown in Fig. 1 clearly shows the ability of the method to assess the analyte in the presence of other excipients.

Fig. 1: Optimized Chromatogram of Tadalafil and Dapoxetine

Linearity and Range:

Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 20-100μg/ml for Tadalafil and 60-300μg/ml for Dapoxetine. A linear relationship was established at these ranges between Area under the peak (AUP) and concentration. Good linearity was proved by high values of coefficient of determinations (Fig.2 and Fig.3). The results were tabulated in Table 2.
TABLE 2: Linearity data of Tadalafil and Dapoxitine

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration of Tadalafil (µg/ml)</th>
<th>Peak area</th>
<th>Concentration of Dapoxitine (µg/ml)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>61652</td>
<td>60</td>
<td>352575</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>129447</td>
<td>120</td>
<td>713850</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>189375</td>
<td>180</td>
<td>1082534</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>244442</td>
<td>240</td>
<td>1408995</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>309206</td>
<td>300</td>
<td>1792376</td>
</tr>
</tbody>
</table>

Fig.2. Linearity graph of Tadalafil

Fig.3. Linearity graph of Dapoxitine

Limit of Detection (LOD)/Limit of Quantitation (LOQ):
The LOD was determined on the basis of signal to noise ratios and was determined using analytical response of three times the background noise. LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. Both LOQ and LOD were calculated on the peak area using the following equations:

LOQ = 10 x N/B

LOD = 3 x N/B

The limit of detection and limit of quantification were evaluated by serial dilutions of Tadalafil and Dapoxitine stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Tadalafil and Dapoxitine was found to be 0.42 µg/mL and 0.27 µg/mL, respectively, and the LOQ value 1.386 µg/mL and 0.89 µg/mL, respectively.

Precision:

System Precision:

System Precision was carried to ensure analytical system is working properly. One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2).
**Method Precision (Repeatability):**

Precision is expressed as the closeness of agreement between a series of measurements obtening from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of sample preparation of Tadalafil (60 μg/mL) and Dapoxitine (180 μg/mL) have been analyzed by injecting them into a HPLC column on the same day and on consecutive days. The results of precision are given in Table 4.

**Table 4: Method Precision data for Tadalafil and Dapoxitine**

| S. No | Tadalafil | | | | | Dapoxitine | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration (µg/ml) | Peak Area | % Assay | Concentration (µg/ml) | Peak Area | % Assay |
| 1 | 60 | 186186 | 98.8 | 180 | 1055440 | 100.22 |
| 2 | 60 | 184635 | 101.6 | 180 | 1060313 | 98.04 |
| 3 | 60 | 188760 | 98.4 | 180 | 1050116 | 100.29 |
| 4 | 60 | 188378 | 101.2 | 180 | 1061756 | 101.20 |
| 5 | 60 | 189416 | 100.9 | 180 | 1076488 | 101.24 |
| 6 | 60 | 191859 | 98.6 | 180 | 1095045 | 99.05 |
| Average | 188205.7 | 99.9 | Average | 1066526.3 | 100 |
| SD | 2528.9 | 1.5 | SD | 16529.5 | 1.3 |
| %RSD | 1.3 | | %RSD | 1.5 | |

**Accuracy:**

The percentage recovery was calculated by preparing standard drug concentrations of Tadalafil and Dapoxitine with concentration levels of 50%, 100% and 150%. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Tadalafil and Dapoxitine was achieved between 97.44-100.35 ± 0.753% and 96.92-100.23±0.327. The results are given in Tables 5,6.

**Table 5: Recovery data of Tadalafil**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Amount added (µg/ml)</th>
<th>Amount found (µg/ml)</th>
<th>%Recovery</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1:50%</td>
<td>30</td>
<td>29.6</td>
<td>98.66</td>
<td>Mean=97.44</td>
</tr>
<tr>
<td>S2:50%</td>
<td>30</td>
<td>29.2</td>
<td>97.33</td>
<td>S.D=1.16</td>
</tr>
<tr>
<td>S3:50%</td>
<td>30</td>
<td>28.9</td>
<td>96.33</td>
<td>%RSD=1.1</td>
</tr>
<tr>
<td>S4:100%</td>
<td>60</td>
<td>60.04</td>
<td>100.06</td>
<td>Mean=99.76</td>
</tr>
<tr>
<td>S5:100%</td>
<td>60</td>
<td>59.42</td>
<td>99.03</td>
<td>S.D=0.63</td>
</tr>
<tr>
<td>S6:100%</td>
<td>60</td>
<td>60.12</td>
<td>100.2</td>
<td>%RSD=0.6</td>
</tr>
<tr>
<td>S7:150%</td>
<td>90</td>
<td>90.54</td>
<td>100.6</td>
<td>Mean=100.35</td>
</tr>
<tr>
<td>S8:150%</td>
<td>90</td>
<td>90.28</td>
<td>100.311</td>
<td>S.D=0.22</td>
</tr>
<tr>
<td>S9:150%</td>
<td>90</td>
<td>90.14</td>
<td>100.15</td>
<td>%RSD=0.2</td>
</tr>
</tbody>
</table>
Robustness:
Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (1.0 ± 0.2 mL), column temperature (30 ± 5°C), mobile phase ratio of the mobile phase. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION:
RP-HPLC method for the simultaneous estimation of Tadalafil and Dapoxitine in their combine dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Tadalafil and Dapoxitine in the range of 20-100µg/ml for Tadalafil and 60-300µg/ml for Dapoxitine with correlation coefficients \( r^2=0.999 \). The percentage recoveries of Tadalafil and Dapoxitine were achieved in the range of 96.92-100.35%. which was with in the acceptance criteria. The percentage RSD was NMT 2% which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.
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