UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF FIRST DERIVATIVE METHOD FOR SIMULTANEOUS ESTIMATION OF SILDENAFIL CITRATE (SIL) AND ASPIRIN (ASP) IN BULK AND TABLET DOSAGE FORM

Avani P. Khristi, Dr. R. B. Mardia, Dr. B. N. Suhagia
Faculty of Pharmacy/ Dharmsinh Desai University, Nadiad- Gujarat.

ARTICLE INFO
Article history
Received 24/08/2015
Available online 30/09/2015

Keywords
Spectrophotometric, Sildenafil.

ABSTRACT
In the present research work, a simple, accurate, precise, reproducible and cost effective first derivative UV Spectrophotometric method developed and validated for the simultaneous estimation of Sildenafil Citrate and Aspirin in bulk as well as tablet dosage form. The solution of both the drugs and tablet were prepared in Methanol. First derivative quantitative determination of these drugs was performed at 255 nm for Sildenafil Citrate at zero crossing point (ZCP) of Aspirin and at 291nm for Aspirin at zero crossing point (ZCP) of Sildenafil citrate. This method obeys Beer-Lambert’s law in concentration range of 5-30 µg/mL and 10-80 µg/mL for Sildenafil Citrate and Aspirin respectively. Co-efficient of correlation were found to be 0.998 for both the drugs. The % RSD were not more than 2.0 % which indicates good intermediate precision. The values LOD and LOQ were 0.588µg/mL and 1.782µg/ml for Sildenafil Citrate and 1.08µg/ml and 3.27µg/ml for Aspirin respectively. Percentage estimation of Sildenafil Citrate and Aspirin in tablet dosage form were found to be 101.00 % and 99.47% respectively.

Please cite this article in press as Avani P.Khristi et al. Uv Spectrophotometric Method Development And Validation of First Derivative Method For Simultaneous Estimation of Sildenafil Citrate (Sil) And Aspirin (Asp) In Bulk And Tablet Dosage Form. Indo American Journal of Pharmaceutical Research.2015;5(09).

Copy right © 2015 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION
Sildenafil citrate is a selective inhibitor of phosphodiesterase type 5 enzyme (PDE5). Chemically it is 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine Citrate which is extensively used for the treatment of erectile dysfunction (ED). Aspirin is a Cyclooxygenase Inhibitors (COX-1 & COX-2). Chemically it is 2-Acetoxybenzenecarboxylic acid having a mechanism by dilating blood vessels in vivo, probably through direct effect on vascular smooth muscle. Therefore, the vasodilator action of aspirin which dilates the blood vessels and increase the blood flow to the smooth muscles which gives synergistic effect to Sildenafil Citrate in patients with ED. Sildenafil Citrate is official in IP & USP. Aspirin is official in IP, BP, USP & JP.

Figure 1: Chemical structure of Sildenafil Citrate.

Figure 2: Chemical structure of Aspirin.

The combination of these two drugs is in demand However there is no such combination product available commercially. The Simultaneous estimation method development and validation has been carried out for such combination dosage form where the combined formula / Cocrystals of the both the drugs has been developed and necessity for the testing. Studies shows that the co-crystallization enhanced the solubility of both the drugs and also providing synergistic effect in the patients suffering from ED.

In the present study, the first derivative zero crossing applied for the simultaneous analysis of the co-crystals and a tablet formulation containing Sildenafil Citrate and Aspirin as the first derivative method allows for the selection of the defined analytical wavelengths of highest value due to the presence of lot of maxima and minima and provide a high sensitivity and accuracy.

MATERIALS AND METHODS
Sildenafil citrate was kindly gifted from Intas Pharmaceuticals Ltd., Acetyl Salicylic Acid (Aspirin – P.No.0250), Co-crystals (Sildenafil Aspirin cocrystals developed in Laboratory of Faculty of Pharmacy, DDU), Methanol, Whatman filter paper no. 41 (Whatman International Ltd., England), Tablet formulation containing Sildenafil Aspirin Co-crystals was prepared in the laboratory of Faculty of Pharmacy, Dharmsingh Desai University, Nadiad, Gujarat.

Instrumentation
UV-visible double beam spectrophotometer, Thermo-scientific 201 with spectral bandwidth of 1nm, and a pair of 1mm matched quartz cells was used.

Preparation of Standard Solutions
Preparation of standard stock solution of Sildenafil Citrate
The standard stock solution of Sildenafil Citrate was prepared by accurately weighed 10 mg of Sildenafil citrate transferred to a 100 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (100 µg/mL).

Preparation of standard stock solution of Aspirin
The standard stock solution of Aspirin was prepared by accurately weighed 10 mg of Aspirin transferred to a 100 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (100µg/mL).
Preparation of Calibration Standards

Calibration standards for Sildenafil Citrate

The Calibration curve for the Sildenafil Citrate was constructed using different concentrations of standard solutions ranging from 5-30 µg/ml. Working standard solutions of Sildenafil citrate (0.5, 1, 1.5, 2, 2.5, 3 mL) were transferred in a series of 10 mL volumetric flasks and diluted up to the mark with methanol (5, 10, 15, 20, 25, 30 µg/mL).

Calibration standards for Aspirin

Calibration curve for Aspirin was constructed using different concentrations of Standard solutions ranging from 10-80 µg/ml. Working standard solution of Aspirin (1, 2, 3, 4, 5, 6, 7, 8 mL) were transferred in a series of 10mL volumetric Flasks and dilute up to mark with methanol (10, 20, 30, 40, 50, 60, 70, 80µg/ml).

Preparation of Sildenafil Citrate and Aspirin Test solution:

The test solution was prepared by standard IP method where twenty tablets were weighed; average weight was calculated and finely powdered. Tablet powder equivalent to 10 mg Sildenafil citrate and 30 mg Aspirin was weighed and transferred to 100 ml volumetric flask containing methanol (50 ml).

The flask was shaken, sonicated for 15 minutes, volume was made up to mark with methanol and filtered through Whatman filter paper no. 41 (100 µg/mL SildenafilCitrate and 300µg/mL Aspirin). An aliquot (1 mL) was taken in 10 mL volumetric flask and volume was made up to the mark with methanol (10 µg/mL Sildenafil citrate and 30 µg/mL Aspirin).

METHOD DEVELOPMENT

Selection of analytical wavelength

The standard solutions of Sildenafil citrate (5-30 µg/mL) and Aspirin (10-80µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra obtained were then processed to obtain first order derivative spectrums and overlaid. At 291 nm Sildenafil Citrate showed zero absorbance and Aspirin showed reasonable absorbance, while at 255 nm Aspirin showed zero absorbance and Sildenafil Citrate showed reasonable absorbance so these two wavelengths were selected for measurement of respective drug.

Preparation of Calibration Curve of Sildenafil Citrate

Aliquots of Standard solution of Sildenafil Citrate (0.5, 1, 1.5, 2, 2.5, 3 mL) were transferred in a series of 10 mL volumetric flasks and diluted up to the mark with methanol (5,10, 15, 20, 25, 30 µg/mL).

The absorbance was measured at 255 nm (zero crossing point -ZCP for Aspirin) and plotted against concentrations. The linearity was observed in the concentration range of 5-30 µg/ml of Sildenafil Citrate.

Preparation of Calibration Curve of Aspirin

Aliquots of Standard solution of Aspirin (1, 2, 3, 4, 5, 6, 7, 8 mL) were transferred in a series of 10mL volumetric flasks and dilute up to mark with methanol (10, 20, 30, 40, 50, 60, 70, 80µg/ml).

The absorbance was measured at 291 nm (zero crossing point –ZCP for Sildenafil Citrate) and plotted against concentrations. The linearity was observed in the concentration range of 10-80 µg/ml respectively for Aspirin.

Figure 1: Overlain zero order spectra of standard solution of Sildenafil citrate (10 µg), Aspirin (10 µg).
METHOD VALIDATION

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guideline Q2 (R1).

Linearity and Range

Calibration curve constructed was linear over the selected range of 5-30μg/ml for Sildenafil Citrate and 10-80μg/ml for Aspirin at λmax of 255 nm and 291 nm respectively. Each concentration was repeated three times. The assays were performed according to experimental conditions and the linearity of the calibration graphs.

Table No. 1: Optical characteristics of Sildenafil Citrate & Aspirin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sildenafil Citrate</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (nm)</td>
<td>255nm</td>
<td>291 nm</td>
</tr>
<tr>
<td>Linearity range (μg/ml)</td>
<td>5-30</td>
<td>10-80</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9960</td>
<td>0.9960</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = -0.013x + 0.02</td>
<td>y = -0.000x - 0.002</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>-0.013</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the developed method was confirmed by doing recovery study of both drugs as per ICH guidelines at three different concentration levels- 50%, 100%, 150% by replicate analysis (n=3). This study was performed by addition of known amounts of both pure drugs to a known concentration of the tablets (oral dispersible tablet containing sildenafil aspirin co-crystals formulated in the laboratory). The amount of standard recovered was calculated in the terms of mean recovery with the percent Relative Standard Deviation (% RSD).

Table No. 2: Results of Recovery Studies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Amount added (%)</th>
<th>Sildenafil Citrate % recovery ± S.D.</th>
<th>% RSD</th>
<th>Aspirin % recovery ± S.D.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>95 ±1.48</td>
<td>1.57</td>
<td>97.0 ± 0.64</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>96 ±0.47</td>
<td>0.49</td>
<td>103.9±0.47</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>96 ±0.94</td>
<td>0.97</td>
<td>102.9±0.01</td>
<td>1.55</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>98 ±0.43</td>
<td>0.44</td>
<td>100±0.0154</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

Precision:

Precision was determined by studying the repeatability which indicates the precision under the same operating conditions over a short interval time. The experiments were repeated for six times for precision. The developed method was found to be precise for intraday and inter day precision on the basis of % RSD values for both drugs.
Table No. 3: Results of Intraday Precision Studies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. (µg/mL)</th>
<th>Sildenafil Citrate Intra-day precision (n=3)</th>
<th>Conc. (µg/mL)</th>
<th>Aspirin Intra-day precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD % RSD</td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1.</td>
<td>10</td>
<td>-0.012±0.00017 1.46</td>
<td>40</td>
<td>-0.0079±0.00010 1.26</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>-0.025±0.00050 1.97</td>
<td>60</td>
<td>-0.0167±0.00025 1.50</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>-0.036±0.00058 1.58</td>
<td>80</td>
<td>-0.023±0.00011 0.50</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

Table No. 4: Results of Inter-day Precision Studies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. (µg/mL)</th>
<th>Sildenafil Citrate Inter-day precision (n=3)</th>
<th>Conc. (µg/mL)</th>
<th>Aspirin Inter-day precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD % RSD</td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1.</td>
<td>10</td>
<td>-0.012±0.00025 1.97</td>
<td>40</td>
<td>-0.012±0.00021 1.81</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>-0.026±0.00040 1.50</td>
<td>60</td>
<td>-0.016±0.00028 1.71</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>-0.036±0.00058 1.58</td>
<td>80</td>
<td>-0.0239±0.00001 0.41</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

Table No. 5: Results of LOD and LOQ:

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil Citrate</td>
<td>0.588 µg/mL</td>
<td>1.782 µg/mL</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1.08 µg/mL</td>
<td>3.27 µg/mL</td>
</tr>
</tbody>
</table>

Table No. 6: Results of Tablet Assay.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Label Claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label Claim (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sildenafil Citrate</td>
<td>Aspirin</td>
<td>Sildenafil Citrate</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>150</td>
<td>50.07</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>150</td>
<td>50.21</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>150</td>
<td>50.14</td>
</tr>
<tr>
<td>Mean</td>
<td>50</td>
<td>150</td>
<td>50.14</td>
</tr>
<tr>
<td>SD</td>
<td>0.0099</td>
<td>0.0072</td>
<td>0.99</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The present paper describes the estimation of Sildenafil Citrate and Aspirin in bulk (co-crystals) and tablet dosage form by Simultaneous equation using first derivative UV method. Solubility studies indicated that both drugs shows better solubility and stability in Methanol. The Beer-Lambert’s concentration range was found to be 5-30 μg/ml for Sildenafil Citrate and 10-80μg/ml for Aspirin at 255 nm and 291 nm respectively. Co-efficient of correlation were found 0.998 for both the drugs (Table No. 1) for proposed method. Precision was determined by studying the interday and intraday precision. The standard deviation and Relative Standard deviation (% RSD) were calculated for both drugs. For proposed method % RSD were not more than 2.0 % which indicates good intermediate precision (Table no. 3 and 4). The values LOD and LOQ were 0.588μg/mL and 1.782μg/ml for Sildenafil Citrate and 1.08μg/ml and 3.27μg/ml for Aspirin respectively (Table No. 5). Percentage estimation of Sildenafil Citrate and Aspirin in tablet dosage form were 101.00 % and 99.47% by the proposed method respectively (Table No. 6).

CONCLUSIONS

There is a high demand for the combination drug of Sildenafil Citrate and Aspirin in various dosage forms. As BCS class II drug, Sildenafil citrate has poor water solubility and so to increase the solubility, the drug undergoes various forms e.g. co-crystallization. Aspirin gives synergistic effect to the Sildenafil Citrate. To analyze such combination the first Derivative UV Spectroscopic method was developed and validated for the simultaneous determination of Sildenafil Citrate and Aspirin in Sildenafil Aspirin co-crystals. Proposed method was also applied to analyze the tablet dosage form. Both the co-crystals and tablet dosage form were developed in the laboratory. The results are in good agreement with the label claim. No excipient or solvent interferes in the analysis. The developed method is simple, precise, accurate, reproducible and cost effective. The method can be applied for the routine analysis of sildenafil citrate and Aspirin in combined dosage form. Recommend future research.

ACKNOWLEDGEMENT

The authors are thankful to Intas Pharmaceuticals Ltd., Ahmedabad for providing Sildenafil Citrate free gift samples.

REFERENCES

3. Indian Pharmacopoeia, Published by Indian Pharmacopoeia Commission Govt. of India, Ministry of Health and Family Welfare, New Delhi, volume III; 2010: 2100.
13. Indian Pharmacopoeia, Published by Indian Pharmacopoeia Commission Govt. of India, Ministry of Health and Family Welfare, New Delhi, volume II; 2010: 842,843.

www.iajpr.com

