SPECTROFLUOROMETRIC METHOD FOR THE ESTIMATION OF SITAGLIPTIN PHOSPHATE IN BULK AND PHARMACEUTICAL FORMULATION

Pratik M. Tailor*, Praful P. Dedhiya, Shailesh A. Shah, Dinesh R. Shah

Department of Quality Assurance, Maliba Pharmacy College, Tarasadi, Gopal Vidya Nagar, Gujarat, India.

ABSTRACT
A simple, accurate, sensitive and reproducible spectrofluorometric method was developed and validated for the analysis of Sitagliptin in bulk and pharmaceutical dosage forms. Sitagliptin showed fluorescence in double distilled water having excitation wavelength at 265 nm and emission wavelength at 580 nm. The calibration graph was prepared in the concentration range of 10-50µg/ml and was found to be linear. The correlation coefficient was found to be 0.998. The proposed method was validated and successfully applied for analysis of tablet dosage forms.

KEYWORDS: Sitagliptin, Spectrofluorometric method, Excitation, Emission.

INTRODUCTION
Sitagliptin phosphate(SGP) chemically, (3R)-3-amino-1-{3-(trifluoromethyl)-5,6 dihydro [1,2,4] triazolo [4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one phosphate monohydrate is a novel oral hypoglycemic drug from the dipeptidyl peptidase 4 inhibitors class. Sitagliptin is used for the treatment of the diabetes1. Sitagliptin is not official in any of the pharmacopoeia. Literature survey reveals that few analytical methods like UV -spectrophotometric, spectrophotometric, RP-HPLC, LC MS are available for the estimation of the sitagliptin in plasma, bulk, and pharmaceutical dosage form2-10. This study presents a new spectrofluorometric method for the determination of sitagliptin phosphate in bulk and pharmaceutical formulations.

Fig.1. structure of Sitagliptin Phosphate Monohydrate
**EXPERIMENTAL**

**Apparatus:**
Schimadzu RF-5301 PC Spectrofluorophotometer with RFPC software was utilized in the study.

**Material and methods:**
Sitagliptin phosphate was obtained as a gift sample from Sun Parma, Mumbai. Double distilled water was utilized in the study. Januvia 100mg and 50 mg tablets were obtained from local pharmacy.

**Preparation of the stock solution:**
100 mg of sitagliptin phosphate was accurately weighed and transferred to 100 ml volumetric flask and dissolved in about 25ml of double distilled water. The volume was made up to mark with distilled water to give 1000 µg/ml.

**Preparation of the calibration curve of Sitagliptin Phosphate**
By scanning a suitable standard solution in spectrofluorometer excitation and emission wavelength was determined, shown in fig. 2. Standard solutions for calibration curve were prepared from stock solution. Fluorescence intensity was measured at 265 excitation wavelength. The calibration curve was constructed by plotting the fl.Intensity v/s concentration (µg/ml). Correlation coefficient was calculated. The summary of calibration curve data presented in Table 1.

**Method validation:** The method was validated as per international conference on harmonization (ICH) guidelines. linearity of the method was determined by mean of calibration graph using different concentration mentioned in the table 1. Linearity was evaluated by visual inspection of the calibration graph. The slope and intercept was reported. LOD and LOQ were calculated from the slope and intercept of the calibration graphs.

The accuracy of the method was measured by recovery studies and standard addition method. A known amount of pure drug at three different levels was added to pre-analyzed sample solution and total concentration was determined using spectrofluorometer.

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter- day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same day. The inter-day precision was measured standard solution over three consecutive days; reproducibility of the method was determined by analyzing the intensity of the different seven solutions of the same concentration.
Analysis of the marketed preparations: 20 tablets were weighed and finely powdered. The powder equivalent to 10 mg of sitagliptin was weighed and transferred to the 200 ml of volumetric flask, and volume was made up to 100 with double distilled water resulting into the concentration of 50 µg/ml. The solution was filtered and estimated on the spectrofluorometer.

RESULTS AND DISCUSSION:
Sitagliptin phosphate is soluble in water but partially soluble in methanol, therefore a simple economic method was developed using double distilled water and validated, which reduce the cost of materials, time and labor. The excitation wavelength $\lambda_{ex}$ was found 265 and emission wavelength was found $\lambda_{Em}$. Method was validated according to the ICH guidelines.

![Emission spectrum of the Sitagliptin phosphate.](image)

**Linearity**: Calibration curve was constructed in the range of 10 -50 µg/ml calibration data are shown in table 1. The regression coefficient was found to be 0.998 Shown in fig 3.

![Calibration curve of the Sitagliptin](image)
Table 1. Calibration data

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Concentration (µg/ml)</th>
<th>Florescence intensity ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>78.161±1.51</td>
<td>1.93</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>133.746±2.52</td>
<td>1.88</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>186.637±2.44</td>
<td>1.31</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>237.079±2.10</td>
<td>0.88</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>282.286±2.64</td>
<td>0.93</td>
</tr>
</tbody>
</table>

**LOD and LOQ**

Limit of detection and limit of quantification was calculated by taking the five calibration curves from the equations LOD=3.3*(Std. deviation of the intercept/mean of slopes) and LOQ = 10*(Std. deviation of the intercept/mean of slopes). The LOD for method was found to be 1.75 µg/ml and LOQ was found to be 5.32 µg/ml.

**Precision**

The precision of the method was evaluated by performing several estimation of sitagliptin. The precision of the method was evaluated by the intraday and interday precision. The RSD lies in the range of 0.24 -1.30. Detail results shown in the table 2.

Table 2. Intraday and Interday precision

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Intraday precision</th>
<th>Interday precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fl. intensity Mean ± S.D. (n=3)</td>
<td>% RSD</td>
</tr>
<tr>
<td>10</td>
<td>80.711 ± 0.881</td>
<td>1.09</td>
</tr>
<tr>
<td>20</td>
<td>135.428 ± 0.330</td>
<td>0.24</td>
</tr>
<tr>
<td>30</td>
<td>189.309 ± 0.703</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Accuracy**

The accuracy of the method was evaluated by means of the recovery studies. The % recovery of the drug was calculated. Mean percentage recovery was found to be 99.07% The detail results are shown in the table 3.

Table 3. Accuracy data

<table>
<thead>
<tr>
<th>Concentration from tablet matrix</th>
<th>Amount of sitagliptin recovered</th>
<th>% Recovery</th>
<th>Mean recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration from tablet matrix</td>
<td>Amount of sitagliptin recovered</td>
<td>% Recovery</td>
<td>Mean recovery</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>29.64 ± 0.28</td>
<td>99.15</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>39.69 ± 0.11</td>
<td>98.89</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>49.62 ± 0.20</td>
<td>99.16</td>
</tr>
</tbody>
</table>
Method Application

The method was applied for the assay of the marketed formulation of 50 mg and 100 mg dose. Average % of labeled claim was found to be 98.82 and 98.84 respectively, detail results are shown in table 4.

### Table 4. Analysis of the marketed formulation

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Brand name</th>
<th>labeled claim</th>
<th>% of labeled claim found (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Januvia (merck)</td>
<td>50 mg</td>
<td>98.82</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>100 mg</td>
<td>98.54</td>
</tr>
</tbody>
</table>

CONCLUSION

The developed spectrofluorometric method is simple, accurate, precise and reliable for the estimation of Sitagliptin in bulk and dosage form. The relative standard deviation (RSD) for all parameters indicates the validity of method and assay results are also within the limit so the proposed method can be used for estimation of sitagliptin.

REFERENCES:

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For Correspondence
Pratik Tailor
Email: pratikmtailor@gmail.com