Spectrophotometric determination of Sitagliptin Phosphate in bulk and pharmaceutical formulations

N. S. Disha*, Dr B.M Gurupadhayya
Sree Siddaganga College of Pharmacy, Tumkur, BH Road -572102
Department of pharmaceutical analysis

ABSTRACT

A simple precise, cost effective and reproducible calorimetric method for the determination of sitagliptin phosphate (STG) in bulk and tablets. STG reacts with 2, 4 DNP and forms precipitate and on addition of 0.1N sulphuric acid the precipitate gets dissolve and made up with methanol and shows maximum absorption at 400nm. The method was linear in a concentration range between 2-10µg/ml. The regression line equation was: Y = 0.0248x+0.0082 with a regression coefficient of 0.9967 (n=5). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.139µg/ml and 0.422µg/ml, respectively. The precision was satisfactory; the values of relative standard deviation (RSD) had not exceeded 2%. The average values of recovery study were found to be in the range 98.72 - 108.2%.

Conclusion: The developed method was simple, fast, accurate and precise. It could be applied for routine quality

Key words: Sitagliptine Tablets (STG), 2, 4, DNP, Beer’s Law.

INTRODUCTION

Sitagliptin phosphate (STG) is an oral hypoglycemic agent[1] that blocks selectively the dipeptidyl peptidase 4 (DPP-4) [3] enzyme reduces the breakdown of GLP-1 and increases insulin secretion[2]; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels.

The literature review reveals several methods for determination of sitagliptin in tablets alone or in combination with other hypoglycemic agents. The major reported analytical methods depended on sophisticated instrumental techniques e.g.UV –spectroscopy[4-7],

Molecular formula:1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8- tetrahydro-3-(trifluoromethyl), phosphate

Molecular weight: 523.32 gm

λ max: 222-279nm

Use: Hypoglycemic
Spectrofluorimetric[8], RP-HPLC[9-13], HPTLC[14], UPLC[15], capillary zone electrophoresis [16] and Mass spectroscopy[17-18].

A comprehensive literature research reveals the lack of a spectrophotometric determination of sitagliptine phosphate in bulk and pharmaceutical formulations by using 2,4 DNP. A successful attempt was made to develop accurate, precise and simple method.

MATERIALS AND METHODS

Apparatus: A UV-visible spectrophotometer model 3000+ Lab India with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing samples.

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used throughout. Sitagliptine phosphate pure sample was obtained as a gift sample from Kanvista Formulations, Hyderabad, India.

Reagents preparation

2, 4-Dinitrophenyl hydrazine (2,4-DNP): The reagent solution was freshly prepared by dissolving 0.5%w/v of 2, 4-DNP in 1 ml of concentrated sulphuric acid and diluting to 100 ml with methanol.

Preparation of Stock Solution

A standard stock solution containing mg/ml was prepared by dissolving 100 mg of Sitagliptin Phosphate in 10 ml of methanol, shake well till it dissolves and make up to 100 ml.

Preparation of Working Standard Solution

From the above stock solution, working standard solution was prepared from 2-10µg/ml respectively.

Standard solutions

The marketed tablets form of sitagliptine used in the determination was Januvia 100mg with a labeled strength of 100mg and manufactured by MSD Pharmaceuticals Limited, Pavia, Italy.

Preparation of calibration curve:

Standard solutions of STG in methanol, having final concentrations in the range of 2-10 µg/ml each ml was transferred into a series of 10 ml volumetric flasks. To that 1ml of 2, 4-Dinitrophenyl hydrazine was added it reacts and forms precipitate, and which was dissolved by addition of 1ml of 0.1N sulphuric acid, and the mixture was then gently shaken until the precipitate was dissolved.
the contents were diluted up to 10 ml with methanol. It showed a maximum absorbance at 400 nm against the reagent blank and the calibration curve was plotted between concentration and absorbance shown in Figure 2.

![Calibration curve](image)

**Figure 2 Calibration curve**

**Procedure for pharmaceutical formulations**

Ten tablets were weighed and their contents were mixed thoroughly. An accurately weighed portion of powder equivalent to the 100 mg of sitagliptine phosphate was weighed into a 100 ml volumetric flask containing about 20 ml of methanol. The mixture was shaken thoroughly for about 5-10 min, filtered to remove insoluble matter and diluted to the mark with methanol to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with methanol to obtain a concentration of 100 µg/ml. Then to that solution 1 ml of drug solution and 1 ml of 2, 4-Dinitrophenyl hydrazine was added and forms a precipitate, precipitate was dissolved by addition of 1 ml of 0.1N sulphuric acid. The mixture was then gently shaken until the precipitate dissolves and forms yellow brown color. The contents were diluted up to 10 ml with methanol.

**RESULTS AND DISCUSSION**

**Reaction between STG and 2, 4, DNP:**

Selection of this reagent was based on the higher reactivity of 2, 4-DNP compared to other hydrazine derivatives and the presence of strong chromophore group (Y.R. Sharma 2002) in its structure that enables its use for the colorimetric determination of several aldehydes and ketones. In addition to being specific for carbonyl groups 2, 4-DNP has advantages over other reagents such as dyes in ion-pair spectrophotometry, as not much care is needed regarding the pH of the reaction and there is no need to extract the product formed. Also the hydrazone and hydrazide products are more red shifted than those of other methods. Also the stability of the colored product can be accounted for by the fact that the reaction is stable.
Optimization of parameter

For this method it was found that optimum concentration of sulphuric acid was 0.1N and optimum concentration of 2,4-DNP was 0.5% w/v. The optimum volume was found to be 1.0 ml sulphuric acid and that of 2,4-DNP was 1.0 ml.

Stability of the chromogen

Under the optimum conditions, the reaction between Sitagliptine phosphate and 2, 4-DNP was completed within 2 minutes at room temperature and the absorbance no longer changed after standing for up to 40 minutes. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. Found that the absorbance of the chromogen remains stable for 1 hour.

Quantification

The limits of the Beer’s law, the molar absorptivity and the Sandell’s sensitivity values were evaluated and are given in Table 1. The regression analyses of the Beer’s law plots at their respective max values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $y = bx + c$ (where $y$ is the absorbance of a 1 cm layer, $b$ is the slope, $c$ is the intercept and $x$ is the concentration of the drug in g/ml) obtained by the least-squares method. The results are summarized in Table 1.
Validation of the method

The validity of the method for the assay of sitagliptine was examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer’s law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the method. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure sitagliptine were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference [19]. The results are given in Table 2. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

\[ y = bx + c, \text{ where } x \text{ is the concentration of drug in } \mu\text{g/ml}; \text{ Average of six determinations} \]

Linearity

To establish linearity of the proposed methods, a series of STG solutions of concentration (2-10µg/ml), were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

Precision:

The precision of the method was determined by replicate analysis of six separate solutions of the working standards at two concentration levels of each drug. At two concentrations intraday and inter day precision studies were performed for two consecutive days. Relative standard deviation was calculated and given in table 4.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations three serial dilutions were prepared from independent stock solutions and analyzed. Accuracy was assessed as the percentage relative error and mean % recovery. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug to a known preanalyzed formulation sample and the total concentration was determined using the proposed methods. Recovery values were calculated and represented in table 2. The percentage recovery of the added pure drug was calculated as

\[ \% \text{ recovery} = [(C_t - C_s)/C_a] \times 100, \]

Where
Ct is the total drug concentration measured after standard addition;
Cs drug concentration in the formulation sample;
Ca, drug concentration added to formulation.

Results of recovery study by standard addition method

**Limit of detection (LOD) and limit of quantitation (LOQ):**

The LOD and LOQ for sitagliptine by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 s/S and 10 s/S, respectively, Where S is the slop of the calibration curve and s is the standard deviation of y-intercept of regression equation. The results of LOD and LOQ are given in table 1

**CONCLUSION**

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax</td>
<td>400nm</td>
</tr>
<tr>
<td>Beers law limits (µg/ml)</td>
<td>2-10</td>
</tr>
<tr>
<td>Molar absorptivity (l/mol/cm)</td>
<td>0.2485*10^-4</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.9967</td>
</tr>
<tr>
<td>Sandell’s sensitivity(µg cm)</td>
<td>0.071</td>
</tr>
<tr>
<td>Regression equation (y)</td>
<td>0.0248x+0.0082</td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.0248</td>
</tr>
<tr>
<td>Intercept, c</td>
<td>0.0082</td>
</tr>
<tr>
<td>Relative standard deviation %</td>
<td>0.477</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>1.08</td>
</tr>
<tr>
<td>Limit of quantification(µg/ml)</td>
<td>3.30</td>
</tr>
</tbody>
</table>

Table 2: Analysis of Tablet formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/tab)</th>
<th>% Drug found</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>STG</td>
<td>100</td>
<td>100.6</td>
<td>1.06</td>
</tr>
</tbody>
</table>
Table 3: Recovery study of STG

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Level of addition %</th>
<th>Amount added µg/ml</th>
<th>Amount recovered µg/ml</th>
<th>% RECOVERY</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>STG</td>
<td>80</td>
<td>8</td>
<td>7.9</td>
<td>98.75</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>10.06</td>
<td>100.6</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>12</td>
<td>13.02</td>
<td>108.5</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Table 4: Evaluation of accuracy and precision

<table>
<thead>
<tr>
<th>DRUG</th>
<th>S.NO</th>
<th>LABLE CLAIM</th>
<th>Amount found</th>
<th>% purity</th>
<th>AVERAGE</th>
<th>SD</th>
<th>%RSD (INTERDAY)</th>
<th>%RSD (INTRADAY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STG</td>
<td>1</td>
<td>100mg</td>
<td>99.8</td>
<td>99.8</td>
<td>99.82</td>
<td>0.726</td>
<td>0.243</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>99.02</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>100.05</td>
<td>100.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>101.07</td>
<td>101.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>99.8</td>
<td>99.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>99.02</td>
<td>99.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


19. ICH Q2 R1, Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 June 1995.