Validation and Application of a High-Performance Liquid Chromatography Method for Estimation of Sitagliptin Phosphate from Bulk Drug and Pharmaceutical Formulation

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ABSTRACT:
A gradient LC method was developed for determination and quantitation of Sitagliptin in bulk drug and pharmaceutical formulations. The separation was accomplished on a symmetry reversed phase C18 column, 75 mm x4.6mm I.D., 3.5µm column using mobile phase consist of acetonitrile and 0.03% formic acid at flow rate of 0.3ml/min. The eluents were monitored with a UV detector set at 268 nm as detection wavelength. The investigated validation elements showed the method has acceptable specificity, accuracy, linearity, precision, robustness. This method was found to be linear within range of 100 to 500 µg.ml⁻¹ (r=0.9999). The method could be of use for rapid and routine quality control analysis of Sitagliptin. Thus, the developed method can be used for process development as well as quality assurance of Sitagliptin in bulk drug and pharmaceutical formulations.

KEYWORDS: Sitagliptin phosphate, RP-HPLC, validation, liquid chromatography, quality assurance.

1. INTRODUCTION:
Sitagliptin Phosphate1-4 is described chemically,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl),1,2,4-triazolo[4,3-a]pyrazine phosphate (Fig-1). It is a first dipeptidyl peptidase-4 (DPP-4) inhibitor class approved by the Food and drug administration (FDA).5 The benefit of this medicine is its lower side effects (e.g. less hypoglycemic, less weight gain) in the control of blood glucose values. Literature survey reveals that the RP-HPLC6 method reported for the determination of Sitagliptin phosphate, and LC-MS7-10 methods were reported for the determination of sitagliptin phosphate in plasma and urine of humans, rats and dogs. The aim of this study was to develop a new simple, sensitive and specific RP-HPLC method for estimation of Sitagliptin phosphate. So far, no simple, fast and precise liquid chromatographic method has been reported in the literature for determination of sitagliptin in drug substance and drug product.

On this background, the present study was carried out to determine sitagliptin in the drug substance and drug product, and to validate the method as per ICH guidelines. The objective of the present study aimed at the development of a simple, fast and sensitive RP-HPLC method for the determination of sitagliptin in bulk drug and formulation.

2. MATERIAL AND METHOD:
2.1 Reagents:
HPLC grade and acetonitrile procured from Merck (India) were used. Formic acid was of AR grade, procured from Merck (India). HPLC grade water obtained from Millipore system (Millipore Inc., USA) was used throughout the analysis. The investigated sample, Sitagliptin phosphate was obtained from Matrix Pvt ltd, as a gift sample. Drug
product, Januvia is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus was purchased from local market.

2.2 HPLC (analytical) instrumentation and operating conditions:
A Shimadzu Instrument UFLC LC-20 AD Liquid chromatography system having Shimadzu SPD-20 A UV/VIS Detector having SSQD (Slow suction quick delivery) Pumping method. A LC method containing mobile phase 60 volume of Acetonitrile and 40 volume of 0.03% formic acid was used at a flow rate of 0.3 ml/min. Symmetry C18 (75mm×4.6mm) 3.5µm was used for separation. The injection volume was 10µL. The data were acquired at 268 nm for 10 min. All the determinations were performed at 25°C column temperature.

2.3 Preparation of Standard Solutions:
Standard stock solution containing Sitagliptin phosphate was prepared by dissolving 100 mg of Sitagliptin phosphate and transferred to 100 ml volumetric flask. Add 50 ml of mobile phase and sonicate and make up the volume up to 100 ml with mobile phase. Dilute further 3 ml of above solution to 10 ml with mobile phase.

2.4 Preparation of Sample Solutions:
A total of 20 tablets were accurately weighed and triturated with glass mortar and pestle. Weigh accurately powder sample equivalent to 100 mg Sitagliptin phosphate into 100 ml volumetric flask, Add sufficient mobile phase, sonicate, cool and make up volume up to mark with mobile phase. Dilute further 3.0 ml of above solution to 10.0 ml with mobile phase.

2.5 Optimization of chromatographic conditions:
The method for the estimation of Sitagliptin phosphate is developed using different mobile phases at different pH. Because of high dependence on mobile phase composition; the attempts to improve selectivity, peak shapes and to reduce the retention times by altering buffer pH, acetonitrile and methanol composition were not successful. Finally, a sharp elution of peaks and good selectivity with variable mixtures of 60 volume of acetonitrile and 40 volume of 0.03% formic with UV detector set at 268 nm were found to be ideal mobile phase for the determination of Sitagliptin phosphate at 25°C. A typical chromatogram of sitagliptin standard was given in Fig. 2. The system suitability factors were described in Table 1. The optimized method was validated as per ICH guidelines.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Injection</th>
<th>Time(Min)</th>
<th>Area</th>
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<tbody>
<tr>
<td>1</td>
<td>Sitagliptin-1</td>
<td>1.803</td>
<td>2639164</td>
</tr>
<tr>
<td>2</td>
<td>Sitagliptin-2</td>
<td>1.802</td>
<td>2651847</td>
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<tr>
<td>3</td>
<td>Sitagliptin-3</td>
<td>1.805</td>
<td>2600412</td>
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<tr>
<td>4</td>
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<td>2586704</td>
</tr>
<tr>
<td>5</td>
<td>Sitagliptin-5</td>
<td>1.810</td>
<td>2599691</td>
</tr>
<tr>
<td>6</td>
<td>Sitagliptin-6</td>
<td>1.810</td>
<td>2605565</td>
</tr>
</tbody>
</table>

Mean 1.806 2613897
% RSD 0.19 0.98

3. Validation:
3.1. Specificity (selectivity):
The data on degradation studies revealed that the degradation products were well separated from the sitagliptin and the peak purity data of sitagliptin indicated that is was spectrally pure.

3.2. Linearity:
The linearity of peak areas versus different concentrations was evaluated for sitagliptin using five levels ranging from 100µg/ml to 500µg/ml. The linear regression data for all the components tested were presented in table-2. The data shown in is confirmed the detector response at 268 nm were linear over the ranges studied for all components.

3.3. Precision:
System precision was verified using diluted standard solution, which was analyzed for six times and R.S.D. of sitagliptin peak areas was evaluated and found to be 0.38%. Precision of the method was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing six separate sitagliptin sample solutions that were prepared at specification level. The R.S.D. was found to be less than 2.0. The data on studies is given in Table 3.

3.4. Accuracy (recovery):
Accuracy of the method for all the related substance was determined by analyzing sitagliptin sample solutions spiked with all the related substances at three different concentration levels of 80, 100 and 120% of each in triplicate at the specified limit. The recovery of all these related substances were found to be in-between the predefined acceptance criterion of 98.0–102.0% and the data is given in Table 4.

3.5. Stability of analytical solution:
To determine the stability of sample solution, the sample solutions of sitagliptin spiked with related substances at specified level were prepared and analyzed immediately after preparation and after different time intervals initial, 8h, 16h and24h. The results from these studies indicated, the sample solution was stable at room temperature and stable for at least 24 h at room temperature.
3.6. Robustness:
To evaluate the robustness of the method, the influence of small and premeditated alteration of analytical parameters on the quantification of the related substances and selectivity was studied. The parameters selected were mobile phase composition (±2% of gradient composition), pH of the mobile phase (±0.05 units), flow rate and wavelength (±2 nm). Only one parameter was changed while the others were kept unaltered. The mean and R.S.D. for each related substance were evaluated. The difference between the mean values from the repeatability mean results is found to be below 2.0%. Therefore the test method is robust for the quantification of sitagliptin.

4. DISCUSSION:
The present study was aimed at developing a sensitive, precise and accurate RP-HPLC method for analysis of sitagliptin phosphate in bulk drug and in pharmaceutical dosage forms. In order to achieve optimum separation of the component peaks, mixture of acetonitrile: formic acid (60:40 v/v) as mobile phase at flow rate of 0.3 ml/min. on C18 stationary phase was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for sitagliptin phosphate was 1.809 min. The peak areas of Sitagliptin phosphate were reproducible as indicated by low coefficient of variation. A good linear relationship (r=0.9999) was observed between the mean values from the repeatability mean results is found to be below 2.0%. Therefore the test method is robust for the quantification of sitagliptin.

5. CONCLUSION:
In summary, we developed and validated simple, sensitive and reproducible and hence can be used in routine for estimation of Sitagliptin phosphate in bulk as well as in pharmaceutical preparations. The RSD for all parameters was found to be less than two, which indicates the validity of method.

6. ACKNOWLEDGEMENT:
The authors are thankful to Dr A D.Taranalli. Principal KLEU’s college of Pharmacy, Belgaum for providing facilities and encouragement.

7. REFERENCES: