



DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE QUANTITATIVE ESTIMATION OF SITAGLIPTIN PHOSPHATE IN BULK AND TABLET DOSAGE FORM

Raja Abhilash Punagoti^{*1}, Rita Mourya²

¹Department of Pharmaceutical Analysis, Sri Kakatiya Institute of Pharmaceutical Sciences, Unikicherla, Warangal, Telangana, India

²Department of Pharmaceutical Chemistry, College of Pharmacy, SAM Global University, Bhopal, Madhya Pradesh, India

*Corresponding author: dr raja abhilash@gmail.com, dr ritamourya15@gmail.com

ABSTRACT

A novel very rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of Sitagliptin phosphate in bulk and tablet dosage form. It was resolved by using a mobile phase of Acetonitrile and Phosphate buffer in the ratio of 60:40 v/v at a flow rate of 1.0 mL/min. using UV-Visible detector at the wavelength of 263 nm for quantification. Efficient separation was achieved for Sitagliptin on used Cosmosil C₁₈ (100 × 2.1 mm, 5µm). The retention time of Sitagliptin was 2.37 min. The calibration graphs were linear and the method showed excellent recovery for tablet dosage form. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

Keywords: Sitagliptin phosphate, HPLC, New method development, Validation.

1. INTRODUCTION

Sitagliptin phosphate is an oral anti hyperglycemic of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. This enzyme-inhibiting drug is used either alone or in combination with other oral anti hyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus type 2. Sitagliptin works to competitively inhibit the enzyme DPP-4. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the pancreas. This drives blood glucose levels towards normal.

7-[(3R)-3-amino-1-oxo-4-(2,4, 5- trifluorophenyl) butyl]-5,6,7, 8 tetra hydro-3-(trifluoromethyl)-1, 2, 4-triazolo [4, 3-a] pyrazine phosphate (1:1) monohydrate [1]. The structure of the drug is shown in fig. 1 [2]. The solubility of the drug was slight in methanol, very slightly soluble in ethanol acetone and acetonitrile and insoluble in isopropanol and isopropylacetate. The PKa value of drug is 7.7 [3].

Literature survey reveals the availability of various analytical methods for the analysis of Sitagliptin in biological samples by RP-HPLC [4, 5] and few

Spectrophotometric methods were also available for estimation of Sitagliptin in bulk and pharmaceutical dosage form [6, 7]. There was only one RP-HPLC method is also available for this Sitagliptin formulation. The reported RP-HPLC method was not economical in terms of mobile phase composition, flow rates and less efficient [8]. Hence, there is a need to develop an RP-HPLC method for the estimation of Sitagliptin in the tablet formulations. The aim of the present analytical research is to develop simple, precise, accurate, rapid and economical RP-HPLC method for the assay of Sitagliptin phosphate in tablet formulation. The developed new method subjected to the assessment of validation parameters according to ICH [9, 10].

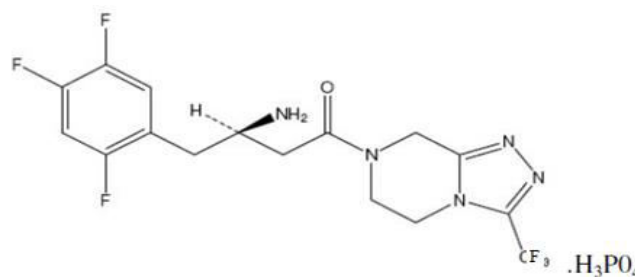


Fig. 1: Structure of Sitagliptin phosphate

2. EXPERIMENTAL

2.1. Materials and Reagents

Sitagliptin phosphate Working Standard was procured from Aurobindo laboratories, Hyderabad, India. Commercially available was Sitagliptin purchased from local pharmacy. Acetonitrile HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

2.2. Chromatographic conditions

Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Chrome- work station HPLC V4.0 software. The chromatographic column used was Cosmosil C₁₈ (100 × 2.1 mm, 5μm). The mobile phase used was Acetonitrile and Phosphate buffer in the ratio of 60:40 v/v at a flow rate of 1.0 mL/min. The detection was monitored at the wavelength of 263 nm. The injection volume was 20.0 μL and the chromatographic runtime of 6 min was used.

2.3. Preparation of solutions

2.3.1. Preparation of Sodium Phosphate buffer

Potassium di hydrogen phosphate (7.0 gm) was dissolved and diluted to 1000mL with milli pore water. the pH was adjusted to 4.0 with ortho phosphoric acid.

2.3.2. Preparation of mobile phase

Mixture of above buffer 400mL (40%) and 600 mL of acetonitrile (60%) was taken and degassed in ultrasonic water bath for 5 minutes. The resultant was filtered through 0.45 μ filter under vacuum filtration.

2.4. Preparation of the Sitagliptin Standard and sample Solution

2.4.1. Standard Solution Preparation

Ten mg of Sitagliptin working standard was transferred into a 10 mL volumetric flask and 1mL of diluent was taken, 9ml of mobile phase was added and then sonicated to dissolve completely and the volume was made up to the mark with the same solvent (Stock solution). Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45μm filter.

2.4.2. Sample Solution Preparation

10mg of Sitagliptin working standard was transferred into a 10 ml volumetric flask and 1mL of diluent was taken, 9ml of mobile phase was added and then sonicated to dissolve completely and the volume was made up to the

mark with the same solvent (Stock solution). Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45μm filter.

2.5. Method validation

2.5.1. Precision

The precision of the method was evaluated by carrying out five independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

2.5.2. Intermediate Precision / Ruggedness

2.5.2.1. Intra-day precision

The precision of the assay method was evaluated by carrying out five independent assays of Sitagliptin (50, 100, 150% i.e. 5.0, 10.0, 15.0 μg/mL) test samples against qualified reference standard. The percentage of RSD of five assay values was calculated.

2.5.2.2. Intermediate precision (inter-day)

Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the five samples of Sitagliptin against qualified reference standard. The percentage of RSD of five assay values was calculated. The %RSD for the area of five replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3. Accuracy

Recovery of the assay method for Sitagliptin was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte's concentration. Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

2.5.4. Linearity

Test solutions were prepared from stock solution at 5 concentration levels (20, 30, 40, 50, and 60μg/mL). The peak area vs. concentration data was treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999).

2.5.5. Limit of Detection (LOD) Limit of Quantification (LOQ)

LOD and LOQ were determined at signal to noise ratios

of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

2.5.6. Robustness

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

3. RESULTS AND DISCUSSION

3.1. Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. At 60:40 (solvent:buffer) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 60:40 (solvent: buffer) in an isocratic mobile phase flow rate. The typical chromatogram obtained for Sitagliptin from final HPLC conditions are depicted in Fig.2.

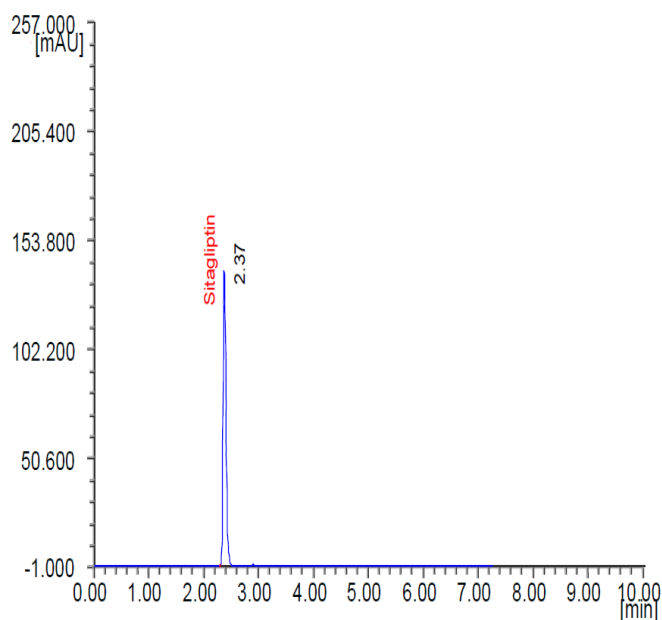


Fig. 2: typical chromatogram of Sitagliptin by proposed method

3.2. Method validation

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows:

3.2.1. System suitability

The system suitability results for the proposed HPLC method are Tailing factor Obtained from the standard injection is 1.4. Theoretical Plates Obtained from the standard injection is 7582.4. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2. Precision

The % R.S.D. of Sitagliptin assay during the method precision was found to be 0.45%, indicating good precision of the method. The results are summarized in table 1.

Table 1: Results of precision

Concentration	Peak area
INJECTION 1	60321.3
INJECTION 2	60368.4
INJECTION 3	60595.1
INJECTION 4	60431.7
INJECTION 5	60875
INJECTION 6	60988.4
Average	60596.65
Standard Deviation	277.8753
%RSD	0.458565

3.2.3. Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Sitagliptin were 0.049 and 0.15 µg/ml, respectively. Since the LOQ and LOD values of Sitagliptin are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

3.2.4. Accuracy

Percentage recovery of Sitagliptin samples ranged from 100.0% to 101.2% and the mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in table 2.

3.2.5. Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e. 10 - 50 µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.6. Robustness

In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 20 -60 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10%

Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate $\pm 10\%$ and change in the Mobile phase $\pm 10\%$. The results are summarized in table 3.

Table 2: Results of Accuracy

%Concentration (at specification Level)	Mean Peak Area (n=3)	Average % Recovery	Mean Recovery
50%	30547.9	98.73%	98.95%
100%	52943.5	98.55%	
150%	55796.4	99.58%	

Table 3: Results of Robustness

Chromatographic changes	USP Plate Count	USP Tailing
Flow rate(ml/min)		
0.8	7797.18	1.47
1.0	7306.18	1.52
1.2	7335.97	1.40
Change in organic composition in the mobile phase		
50:50	2516.69	1.0
60:40	7326.09	1.48
70:30	2337.26	2.18
UV wavelength(nm)		
261	7523.3	1.47
263	7780.65	1.39
265	7821.12	1.48

3.2.7. Application of the developed method to commercial Sitagliptin tablets

When the developed method was used to analyze a commercial brand of Sitagliptin tablet formulation, the mean recovery of five replicates was 99.69 % with % R.S.D. of 0.45. The % recovery value indicates non-interference from the excipients present in the dosage form.

3.3. Method development and optimization

The main aim of the developed method was to achieve separation and quantification of Sitagliptin using an isocratic mobile phase with HPLC system. Developing a HPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Sitagliptin was adequate at 263 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on Cosmosil C₁₈ (100 × 2.1 mm, 5µm). The mobile phase was Acetonitrile and Phosphate buffer in the ratio of 60:40 v/v at a flow rate of 1.0 ml/min. While developing the HPLC method, basic chromatographic

conditions such as the used Cosmosil C₁₈ (100 × 2.1 mm, 5µm) column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the HPLC column, its stability at the lower pH was taken into consideration to preserve the long life of the column. Most commercial C₁₈ columns are not stable at lower pH on the longer run, thus shortening their life span. Column was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 1.0 ml/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Sitagliptin which eluted at around 2.37 min giving a total run time of 10 min.

4. CONCLUSION

The new, isocratic RP-HPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 2.37 min allows the analysis of a large number of samples in a short period of time and is

therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Sitagliptin in tablet formulations.

Conflict of interest

None declared

5. REFERENCES

1. Herman GA, Stevens C, VanDyck K, Bertgman A, Yi B, et al. *Clin Pharmacol Ther.*, 2005; **78 (6)**:675-688.
2. Bergman AJ, Stevens C, Zhou Y, Yi B, Laethem M, et al. *Clin Ther.*, 2006; **28(1)**:55-72.
3. Miller S, Onge EL. *Ann Pharmacother.*, 2006; **40(8)**:1336-1343.
4. Martin M, Pablos F, Gonzalez A. *Talanta.*, 1999; **49**:453-459.
5. Perez-Ruiz T, Martinez-Lozano C, Tomas V, Carpena J. *J Pharm Biomed Anal.*, 1998; **17**:719-724.
Hirai T, Matsumoto S, Kishi I. *J Chromatogr B.*, 1997; **692**:375-388.
6. Bala SC, Prameela RA. *Int. J. Pharm. Pharm. Sci.*, 2010; **2(4)**:138-142.
7. Jeyabalan G, Narendra N. *J. Appl. Pharma. Science*, 2013; **3(1)**:95-98.
8. Lavanya R, Yunoos. *J. Adv. Pharm. Edu. Res.*, 2013; **3(4)**:475-479.
9. International Conference on Harmonization, ICH Q2 (R1) Guideline on Validation of Analytical Procedures: Text and Methodology 2005.
10. Raja Abhilash P, Venkateshwar Rao J. *Der Pharmacia Lettre.*, 2014; **6(5)**:325-329.