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Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate in Bulk and Its Tablet Dosage Form

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A B S T R A C T

A simple and sensitive reversed-phase liquid chromatographic method has been developed and validated for the estimation of Sitagliptin phosphate in bulk and its tablet dosage form. The separation was carried out on Phenomenex C18 (2) Luna coloum (250 × 4.6mm; 5µm) column at ambient temperature using buffer, 0.1 % perchoric acid in water and acetonitrile (60:40) as eluent. The flow rate was 1.0 ml/min and Sitagliptin phosphate was quantified by absorbance at 210 nm. The retention time of Sitagliptin phosphate was 4.42 min. The percentage recovery was within the range between 99.14 % and 101.3 % for Sitagliptin phosphate. The linear ranges were found in the range of 50µg/ml – 150µg/ml (r^2 = 0.984) of Sitagliptin phosphate. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the proposed method could be successfully employed for routine analysis of Sitagliptin phosphate in pharmaceutical formulations according to ICH guidelines.

Keywords: Sitagliptin phosphate, RP-HPLC, Tablets, Estimation, Validation

A R T I C L E I N F O

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1. Introduction

Sitagliptin phosphate (Fig. 1) chemically, (3R)-3-amino-1-[3-(tri fluoro methyl)-5,6-dihydro [1,2,4] triazolo[4,3-a] pyrazin-7(8H)-yl]-4-(2, 4, 5-tri fluoro phenyl)butan-1-one phosphate hydrate, is an oral anti hyperglycemic agent that belongs to dipeptidyl-peptidase 4 inhibitor class which stimulates glucose-dependent insulin release [1]. Sitagliptin phosphate 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. Literature survey reveals the availability of various analytical methods for the analysis of Sitagliptin in biological samples by RP-HPLC[2, 3] and few Spectrophotometric methods are available for estimation of Sitagliptin in bulk and pharmaceutical dosage form[4-8]. The main purpose of our study was to develop a simple, reliable and economical method to determine Sitagliptin phosphate in a relatively short time with high linearity. Therefore, this study focused on the development of simple and rapid RP- HPLC method which can be employed for the routine analysis of Sitagliptin phosphate in pure drug and pharmaceutical formulations.

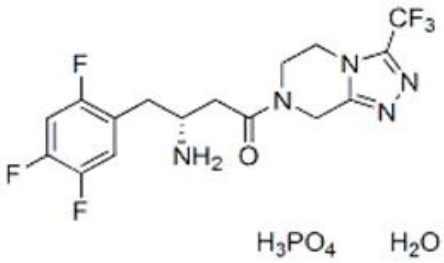

Figure 1: Structure of Sitagliptin phosphate

2. Materials and Methods

Chemicals and reagents

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Perchloric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Sitagliptin phosphate was a gift sample by Bafna Pharmaceuticals Ltd., Chennai – 600 052, Tamil Nadu, India. A commercial tablets containing Sitagliptin phosphate 100mg was procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu 1100 Series, Germany) with UV- Visible dual absorbance detector (PDA), Phenomenex C18 (2) Luna coloum (250 × 4.6mm; 5µm). The mobile phase consisting of buffer, 0.1 % perchoric acid in water and acetonitrile was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 60:40 v/v into the column column at a flow rate of 1.0 ml/min. The detection was monitored at 210nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

64.24 mg of Sitagliptin phosphate working standard was weighed accurately and transferred carefully in 50 ml volumetric flask. About 25 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5ml of above solution was diluted to 50 ml with mobile phase. The resulting solution was mixed and filtered through 0.45 µm membrane filter.

Analysis of Sample Preparation

Twenty tablets containing Sitagliptin were accurately weighed and crushed to fine powder using a glass mortar and pestle. A portion of the power equivalent to about 50 mg of Sitagliptin was weighed and transferred to 50 ml volumetric flask. 25 - 30 ml of mobile phase was then added and sonicated to dissolve the powder completely and the volume was made up with mobile phase. 5 ml of the above stock solution was taken in a 50 ml volumetric flask and diluted up to the mark with mobile phase. Finally the solution was mixed well and filtered through 0.45 µm membrane filter.

Procedure:

About 20 µl each of the test and the standard solutions were injected separately into the chromatograph and the chromatograms were recorded and the responses for the major peaks were then measured. The quantity of Sitagliptin phosphate equivalent to Sitagliptin in mg/ tablet was calculated by using the formula:

Test area Std. wt (mg) 5 50 50 P
---------- x ---------- x-----x-----x-----x-----x Avg wt mg
Std. area 50 50 50 TW 100

Where,

P= Purity of Sitagliptin phosphate working reference standard.

407.32 = Molecular weight of Sitagliptin;

532.32 = Molecular weight of Sitagliptin phosphate monohydrate

3. Results and Discussion

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [9].

System Suitability: It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions were given separately to the system. The system suitability parameters such as retention time, peak area response, number of theoretical plates, tailing factor and their respective mean, standard deviation & %RSD were calculated for the standard drug solutions and mentioned in Table 1. It was observed that all the values are within the limits.

Specificity: The specificity of the HPLC method is illustrated in Fig. 2, where complete separation of Sitagliptin phosphate was noticed in presence of other inactive excipients used in tablet dosage form. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 2.

Linearity and Range

The Linearity of this method was determined at five levels from 50%–150% of operating concentrations for Sitagliptin phosphate and it was shown in Table 3. The plot of peak area of each sample against respective concentration of Sitagliptin phosphate was found to be linear (Figure 3) in the range of 50%– 150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 18404x + 53949 for Sitagliptin phosphate and correlation coefficient of the standard curve was found to be 0.984 for Sitagliptin phosphate. It observed that correlation coefficient and regression analysis are within the limits.

Accuracy:

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, Sitagliptin phosphate was added to pre-analyzed samples at a level from 50% up to 150% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recovery was found to be for Sitagliptin phosphate which demonstrated that the method was highly accurate.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

System Precision

The system precision was established by injecting six replicate injections of working standard solution in to the chromatographic system. System precision data for Sitagliptin phosphate was shown in Table 5. This indicated that system was highly precise.

Method Precision

The method precision was established by injecting six replicate injections of sample solution in to the chromatographic system. Method precision data for Sitagliptin phosphate was shown in Table 5. This indicated that the method was highly precise.

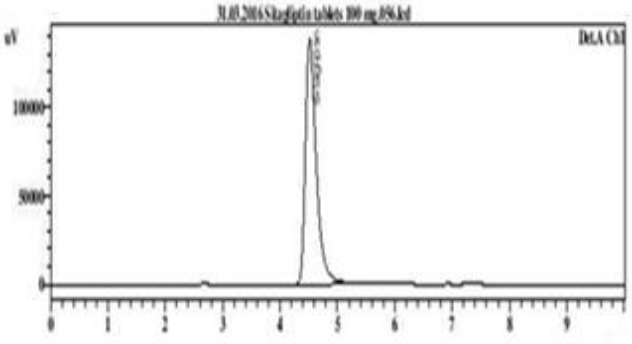

Figure 2: Typical HPLC Chromatogram of Sitagliptin phosphate Tablets

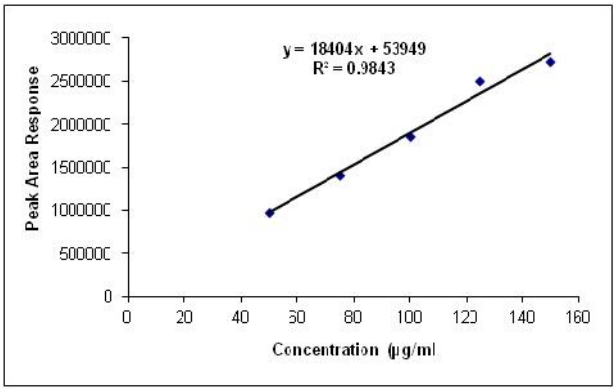

Figure 3: Linearity of response for Sitagliptin phosphate

Table 1: System suitability for Sitagliptin phosphate

S.No	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	4.420	3486544	11171	1.7
2.	Standard -2	4.413	3480631	11230	1.7
3.	Standard -3	4.405	3489551	11250	1.7
4.	Standard -4	4.397	3488321	11184	1.7
5.	Standard -5	4.392	3488814	11198	1.7
Mean		**4.405**	**3486772**	**11207**	**1.7**
Standard deviation		**0.011**	**3607.48**	**32.72**	**0.0**
RSD in %		**0.26**	**0.10**	**0.29**	**0**

Table 2: Specificity for Sitagliptin phosphate

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	3486544
4.	Sample	1	3468040

Table 3: Linearity of response for Sitagliptin phosphate

S.No	Linearity Level (%)	Concentration (µg/ml)	Area**
1.	50	50	975994
2.	75	75	1405472
3.	100*	100	1857020
4.	125	125	2507742
5.	150	150	2725306

*****Operating concentration

**Mean area of duplicate injections

Table 4: Accuracy for Sitagliptin phosphate

S.No.	Spike Level (%)	Area	Average Area	Recovery (%)
1.	50	972629	975375	100.7
2.	50	974715		
3.	50	978782		
4.	75	1395872	1396687	101.0
5.	75	1400138		
6.	75	1394049		
7.	100	1849906	1852936	99.92
8.	100	1848582		
9.	100	1860321		
10.	125	2488477	2495252	99.14
11.	125	2503940		
12.	125	2493340		
13.	150	2718387	2718646	101.3
14.	150	2713411		
15.	150	2724138		
Mean				**100.41**
Standard deviation				**0.877**
RSD in %				**0.87**

Table 5: System Precision for Sitagliptin phosphate

S.No.	Name	Retention Time (min)	Area
1.	Standard -1	4.61	1853067
2.	Standard -2	4.61	1849267
3.	Standard -3	4.62	1846546
4.	Standard -4	4.60	1866411
5.	Standard -5	4.61	1848604
6.	Standard -6	4.59	1852323
Mean			**1852703**
Standard deviation			**7137.26**
RSD in %			**0.39**

Table 6: Method Precision for Sitagliptin phosphate

S.No.	Name	Retention Time (min)	Area
1.	Sample -1	4.61	1725764
2.	Sample -2	4.60	1721024
3.	Sample -3	4.59	1714364
4.	Sample -4	4.58	1731667
5.	Sample -5	4.58	1709990
6.	Sample -6	4.58	1731531
Mean			**1722390**
Standard deviation			**8955.97**
RSD in %			**0.52**

4. Conclusion

The proposed study describes a simple reversed phase - HPLC method for the analysis of Sitagliptin phosphate in pure and in pharmaceutical tablets. The method was validated as per ICH guidelines and found to be linear, specific, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of Sitagliptin phosphate in pharmaceutical dosage form without interference.

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6. References

[1] Badyal DK and Kaur J. Sitagliptin: a new class of oral drug for type 2 diabetes. JK Science. 2008; 10(2):93- 98.

[2] Anildubalal. RP-HPLC method for the estimation of Sitagliptin Phosphate in human plasma by protein precipitation technique. *International journal of pharmacy and pharmaceutical sciences*. 2012; 4(2): 691- 694.

[3] Tian Yan Zhou and Wei Lu RP-HPLC method for the Quantitative determination of sitagliptin in rat plasma. *Journal of Chinese Pharmaceutical Sciences*. 2011; (20): 63-69.

[4] Monila. Spectrophotometric method for determination of Sitagliptin Phosphate in bulk and also in pharmaceutical formulations. *Journal of Pharmacy and Biological Sciences*, 2012; 1(6):37-40.

[5] Jeyabalan.G and Narendra Nyola spectrophotometric method for the Determination of sitagliptin in pure and tablet dosage form. *Journal of Pharmaceutical Analysis*, 2012; 1(1): 19-23.

[6] Rolim M.B. UV-spectrophotometric method for the analysis and dissolution studies of sitagliptin phosphate in tablets Latin. *American Journal of Pharmacy*, (2010); 29 (6): 962-967.

[7] Tarkase K. N, Madhuri B. Sarode, Sumit A. Gulve and Ashwini Gawade. UV spectrophotometric method has been developed for Estimation of Sitagliptin phosphate in tablet dosage form. *Der Pharmacia Lettre*. 2013; 5 (3):315- 318.

[8] ICH Harmonised Tripartite Guideline, Q2(R1), Validation of Analytical Procedures: Text and Methodology.International Conference on Harmonisation, Geneva. 2005; 1-13.

[9] Parag Pathade, Md Imran, Vinod Bairag, and Yogesh Ahire. stability indicating UV Spectrophotometric method has been developed for quantitative determination of Sitagliptin Phosphate in bulk and pharmaceutical formulations. *Journal of Pharmacy Research*, 2011; 4(3): 871-873.