RESEARCH ARTICLE

Development and Validation of RP-HPLC method for the estimation of Sitagliptin Phosphate in Bulk and its Tablet Dosage Form

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ABSTRACT

A new simple and precise reverse phase high performance liquid chromatographic method has been developed and subsequently validated for the estimation of Sitagliptin phosphate monohydrate in bulk and its pharmaceutical dosage form. The chromatographic separation was performed by using mobile phase consisting of 0.01M KH_2PO_4: Methanol in the ratio of 50:50 % v/v and the pH 2.5 adjusted with 0.2% orthophosphoric acid. The column used was Zorbax Eclipse XDB C_{18} (150×4.6 mm, 5µ) with flow rate of 0.7 ml/min using PDA detection at 267 nm. The described method was found to be linear over the range of 5-30µg/ml and correlation coefficient was found to be 0.999. The assay of Sitagliptin was found to be 99.89 %. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, reliable, accurate and economical which is useful for the routine determination of Sitagliptin phosphate in bulk and its pharmaceutical dosage form.

Keywords: Sitagliptin phosphate, method validation

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-1and 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.

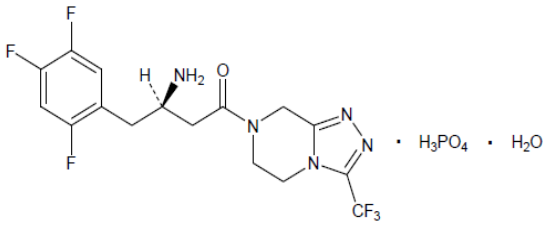

Fig.1. Structure of Sitagliptin phosphate

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7 - [(3*R*) – 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 (1:1) monohydrate.

Literature survey reveals the availability of various analytical methods for the analysis of sitagliptin in biological samples by RP-HPLC.[1,2] and few Spectrophotometric methods are available for estimation of sitagliptin in bulk and pharmaceutical dosage form.[3-7] There is one RP-HPLC method is also available for this sitagliptin formulation. [8] The reported RP-HPLC method was not economical in terms of mobile phase composition, flow rates and less efficient. 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.

MATERIALS AND METHOD

Sitagliptin phosphate gift sample was provided by MSN labs, Hyderabad. A commercial Janumet tablets containing Sitagliptin phosphate 100 mg was purchased from local market, Hyderabad. All other chemicals used were of HPLC grade.

Selection of wavelength of detection

Sitagliptin standard solution of 100 ppm was scanned at 200-400 nm and UV Spectrum was recorded. By observing the spectrum of standard solution, λmax of 267 nm was taken for trails to develop the proposed method. UV-spectra of sitagliptin phosphate is shown in figure.2.

Instrumentation and Chromatographic Conditions

High performance liquid chromatography Agilent 1200 series equipped with PDA detector and Zorbax Eclipse XDB C_{18} (150 mm × 4.6 mm) containing 5 µm particle size column was used. Mobile phase comprising of 0.01M Phosphate buffer: methanol in a ratio 50:50 % v/v at P^H 2.5 adjusted with 0.2 % orthophosphoric acid at a flow rate of 0.7 ml/min and the effluent was detected at 267 nm. The Column temperature was maintained at ambient and the volume of injection is 10 µL.

Preparation of buffer

Phosphate buffer was prepared by dissolving 0.68 gm of potassium dihydrogen orthophosphate in 500 mL of double distilled water. P^H was adjusted to 2.5 with 0.2% ortho phosphoric acid and solution was filtered through 0.45 µ Millipore Nylon filter.

Mobile phase preparation

0.01M Phosphate buffer adjusted to P^H 2.5: methanol in a ratio 50:50 % v/v was taken, sonicated for 15 minutes and filtered through 0.45µ Millipore Nylon filter under Vacuum filtration. The prepared solution was used as Mobile phase.

Diluent Preparation

Diluent 1: Water

Diluent 2: Mobile phase

Preparation of solutions

Standard stock solution:

10 mg of Sitagliptin was accurately weighed and dissolved in diluent1 in a 100 ml volumetric flask and the solution was made up to 100 mL with diluent 1to obtained concentration of 100 µg/ml.

Working Standard solution:

1mL of standard stock solution was pipetted into 10 mL volumetric flask and diluted up to the mark with diluent 2 and filtered through 0.45µ Millipore Nylon filter to obtained concentration of 10 µg/ml.

Sample stock solution:

20 tablets were weighed and average weight of tablet was calculated. The tablets were crushed into a fine powder using mortar and pestle. 42 mg of tablet powder equivalent to 10 mg of Sitagliptin phosphate monohydrate was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Then 70 ml of diluent 1 was added, sonicated for 15 min. and then volume was made up to the mark with the diluent 1 to obtained concentration of 100 µg/ml. Further1 mL of above sample stock solution was pipetted into a 10 mL volumetric flask and diluted up to the mark with diluent 2, filtered through 0.45 µ Millipore Nylon filter to obtained final concentration of 10 µg/ml.

Method validation

The method was validated in terms of the following parameters; linearity, specificity, accuracy, precision, and system suitability parameters as per the ICH guidelines.

Specificity

To determine specificity, a volume of 10µl of working standard, sample and blank solution were injected separately and the chromatograms were recorded and are shown in fig. 3and 4.

Linearity

A series of working standard solutions of Sitagliptin phosphate monohydrate were prepared in the concentration range from 5 to 30(µg/mL) and injected into the chromatographic system. A calibration graph is plotted between concentration of Sitagliptin phosphate monohydrate (µg/mL) and chromatographic peak area (mV). The results are tabulated in Table no.1 and linearity graph was shown in Fig 5.

Accuracy studies

A known amount of working standard at three different levels i.e. 50%, 100%, and 150% were added to pre analyzed sample solution of 100% concentration and injected each three times in to the

chromatographic system. From this % recovery was calculated. Results of the recovery studies are shown in Table no.2

Precision Studies

System precision:

The system precision was established by injecting six replicate injections of working standard solution into the chromatographic system and the results are shown in table no.3

Method precision:

The method precision was established by injecting six freshly prepared sample solutions into the chromatographic and the results are shown in table no.4

Sensitivity

The sensitivity of Sitagliptin by the use of proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The LOQ and LOD were found to be 0.6 µg/ml and 1.9 µg/ml.

Results and Discussions

The proposed method was developed and validated as per the ICH guidelines. Linearity was observed over a concentration range of 5 to 30 µg/ml. System suitability parameters were satisfactory and the theoretical plates were obtained above 2000. Tailing factor was found below 2. %RSD also found below 2%. The assay of Sitagliptin was found to be 99.89% and the low % RSD value confirms the robustness of the method.

Table 1: Data of linearity study

Sl. No.	Concentration (µg/mL)	Peak Area	Statistical analysis
1	5	32500	Slope=6326 Intercept=945.7 Correlation coefficient= 0.999
2	10	66306	
3	15	99201	
4	20	122467	
5	25	156734	
6	30	193693	

Table: 2 Data of Accuracy (Recovery studies)

Sl. No.	Spiked level	Amount of drug from Formulation (µg/mL)	Amount added (µg/ml)	amount found (µg/mL)	% Recovery	Statistical Analysis	
						Mean± SD(n=3)	%RSD
1	50%	10	5	15.10	100.7	100.6 ± 0.374	0.37
				15.02	100.1		
				15.15	101.0		
2	100%	10	10	19.91	99.5	99.84 ± 0.251	0.25
				20.02	100.1		
				19.98	99.92		
				25.22	100.8		
3	150%	10	15	25.34	101.3	100.4 ±0.923	0.97
				24.78	99.14		

Table 3: Data of system precision

Injection	Retention time (min)	Peak area
Injection-1	3.073	66287
Injection-2	3.100	67736
Injection-3	3.173	66286
Injection-4	3.113	66262
Injection-5	3.200	67585
Injection-6	3.173	67591
Mean ± S.D	3.138 ± 0.045	66957 ± 681.3
%RSD	1.43	1.01

Table 4: Data of method precision

Injection	Retention time (min)	Peak area
Injection-1	3.120	71511
Injection-2	3.113	72575
Injection-3	3.113	71676
Injection-4	3.120	71946
Injection-5	3.160	71106
Injection-6	3.120	71851
Mean ± S.D	3.124 ± 0.016	488.1
%RSD	0.512	0.679

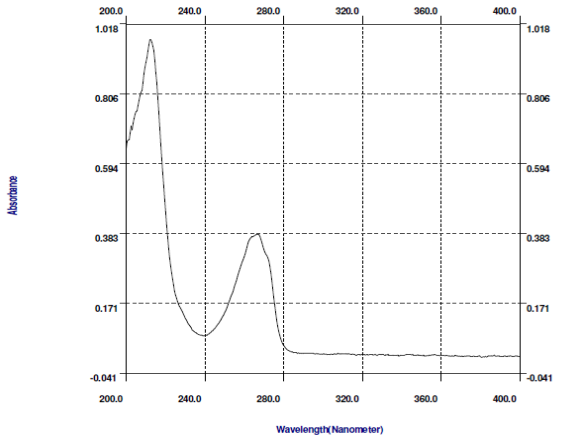

Fig.2: UV spectrum of sitagliptin phosphate in water (100 µg/ml)

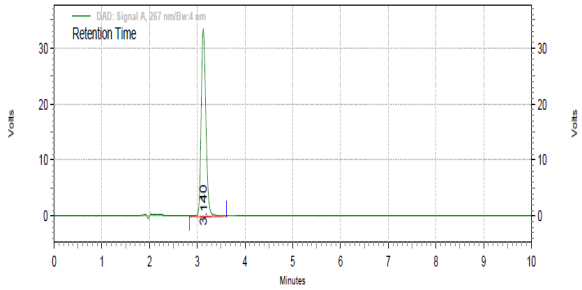

Fig.3 Chromatogram of sitagliptin phosphate in standard solution

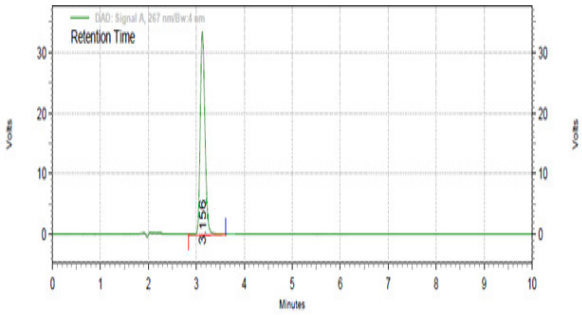

Fig.4: Chromatogram of sitagliptin phosphate in sample solution

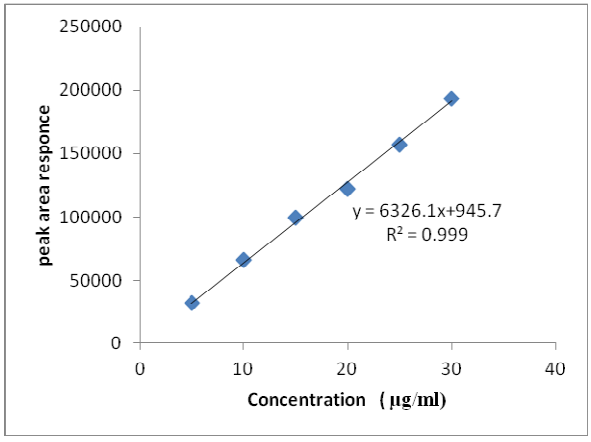

Fig.5: Calibration curve of sitagliptin phosphate (5-30 µg/ml)

Table 5: Summary of Validation Parameters

S. No.	Parameter	Results
1	Limit of Linearity	5-30 µg/ml
2	Regression equation	Y= 6326x+945.7
	Slope	6326
	Intercept	945.7
	Correlation coefficient (r2)	0.999
3	Accuracy (%)	99.8-100.6
4	Precision(%cv)	
	System precision	1.22
	Method precision	0.59
5	LOD(µg/ml)	0.6
6	LOQ (µg/ml)	1.9

CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, linear, robust and specific for quantitative estimation of Sitagliptin phosphate in bulk and its formulation. The proposed RP-HPLC method was cost effective and less time consuming. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence, the present RP-HPLC method is suitable for routine assay of Sitagliptin phosphate in bulk and tablet dosage form in the quality control laboratories.

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