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A SIMPLE VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF SITAGLIPTIN PHOSPHATE IN BULK AND PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT:

A novel, simple and economic reverse phase high performance liquid chromatographic method was developed and validated as per the ICH guidelines for the quantitative estimation of Sitagliptin Phosphate in pharmaceutical tablet dosage form with greater precision and Accuracy. The mobile phase consisted of Methanol: acetonitrile: Orthophosphoric acid (40:55:5). The eluent was monitored at 265 nm, at a flow rate of 1 mL/min and retention time was observed at 10 min. calibration curve as linear over the concentration range of 60-210 ng/ml. RSD% of the determination of precision was <2%. Accuracy of method was determined through recovery studies which were found to be 99.82–101.87%. The LOD and LOQ were found to be 0.05 mg/mL and 0.16 mg/mL respectively. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Sitagliptin Phosphate in bulk and Pharmaceutical tablet dosage forms.

KEYWORDS: Sitagliptine Phosphate, LOD, LOQ, Recovery studies, ICH guidelines.

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INTRODUCTION:

Sitagliptin phosphate is described chemically as 7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl) butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazinephosphate monohydrate is shown as fig1. It has a molecular formula of $C_{16}H_{15}F_6N_5O.H_3PO_4.H_2O$ and a molecular weight of 523.32. Sitagliptin Phosphate is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor for the treatment of type II diabetes which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon like peptide -1(GLP-1) and glucose – dependent insulinotropic polypeptide(GIP). This increase active incretin and insulin levels and decreases glucgon levels and post-glucose–load glucose excursion. Sitagliptin Phosphate is a white to off white, crystalline, non-hygroscopic powder. It is soluble in water and N, N-Dimethyl formamide; slightly soluble in methanol; very slightly soluble in ethanol, acetone and acetonitrile; and insoluble in isopropanol and isopropyl acetate. Several analytical methods based on UV Spectrophotometry, spectroflourimetry, RP-HPLC, HPTLC and LC-MS/MS was reported for the determination of Sitagliptin Phosphate. Although literature survey reveals that various methods were reported in Sitagliptine Phosphate for single estimation and in combination with others drugs. Therefore the main objective of the proposed method was to develop simple, new accurate, precise, sensitive and robust RP-HPLC method for the estimation of Sitagliptin Phosphate in bulk and tablet dosage form and validated as per ICH guidelines.

MATERIALS AND METHODS:

Chemicals and reagents:

Active pharmaceutical ingredient (API) working standard of Sitagliptin phosphate was received as gift samples from Mylan Laboratories Ltd., Hyderabad, India respectively. The Pharmaceutical dosage form used in this study was Januvia tablets manufactured by Merck Co. Ltd. which were purchased from local market. All chemicals and reagents used of HPLC grade.

Instrumental and Chromatographic Conditions:

Schimadzu LC20-AD HPLC system with Rheodyne universal injector 7725 and LC20-AD UV-Visible detector module equipped with Spinchrome software was used. The chromatographic studies were performed using Zodiac C18 column (250 × 4.6 mm) with 5 µm particle size and eluted with mobile phase consisting of methanol: Acetonitrile: 0.1% ortho phosphoric acid (40:55:05) and adjusted pH to 4.1 with triethyl amine at a flow rate of 1 mL/min. The run time was 10 minutes. The mobile phase was filtered through 0.45 µm memberane filter and degassed in ultra Sonicator prior to use10 minutes. Detection was made at 265 nm. The injection volume was 20 µl and all the experiments were performed at ambient temperature.

Preparation of working standard solution:

Accurately weighed 10mg of Sitagliptin phosphate working standard was taken in 10ml volumetric flask, dissolved and diluted to volume with mobile phase and mixed.

Preparation of sample solution:

Exactly 20 tablets were weighed and grinded to fine powder. A quantity of powder equivalent to 10mg of Sitagliptin phosphate was transferred in to a 10ml volumetric flask and dissolved in 7ml of diluent. The solution was sonicated for 15min and shaken for 30min. then diluted to volume with diluent and mixed.

Pipette out 1ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 µ membrane filter. The filtrate was injected for the further analysis.

Method validation:

System suitability:

From the chromatogram obtained for the standard preparation, the column efficiency was determined. The theoretical plates obtained should be not less than 2500 and the tailing factor should be not more than 2.0 and the relative standard deviation of replicate injection should be not more than 2.0%.

Accuracy:

Accuracy of a method is defined as the closeness of a measured value to the true value. To carry out accuracy study of proposed method, the recovery studies were performed by spiking the previously analyzed sample of Sitagliptin phosphate with the known amounts of pure drug at different concentration levels. The spiked levels were 50%, 100% and 150%. The % recovery was calculated three times at each level and the average % recovery was calculated.

Linearity:

Linearity is the ability of the method to respond proportionally to the changes in the concentration of the analyte in a sample. A series of solutions are prepared using Sitagliptin phosphate working standard at concentration levels from 60ppm to 210ppm of target concentration (60, 90, 120, 150, 180 and 210 ppm). The calibration curve was obtained by plotting the concentration against peak area of the each standard solution. The six concentration levels were subjected to regression analysis to calculate calibration equation and correlation coefficient.

Limit of detection and Limit of quantitation:

Limit of detection and Limit of quantitation represents the concentration of analyte that would yield signal to noise ratio of 3:1 and 10:1. The Limit of quantitation is approximately twice than that of Limit of detection.

Precision:

The precision of the method was determined by repeatability (intra-day) and intermediate precision (interday). Repeatability was determined by performing six repeated analysis of the same working solution of Sitagliptin phosphate, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on different days and also by another analyst performing the analysis in the same laboratory (between-analysts).

Robustness:

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. Robustness of developed RP-HPLC method was studied by effect on retention time of Sitagliptin phosphate by changing flow rate (± 0.1 ml/min), composition of organic phase (± 1 %) and pH of mobile phase (±0.1).

RESULTS AND DISCUSSION:

The method utilizing Methanol: Acetonitrile: orthophosphoric acid as mobile phase yielded broad peak, whereas with Methanol: Acetonitrile: orthophosphoric acid tailing was observed with methanol as diluent. Procedure utilizing Methanol: Acetonitrile: orthophosphoric acid as mobile phase with water as diluents also yielded tailing where as with Methanol: Acetonitrile: orthophosphoric acid mobile phase and acetonitrile as diluent sharp peak was obtained. During method development, a number

of variations were tested like Methanol: concentration and flow rate to give a symmetric peak. With a mobile phase Methanol: Acetonitrile: orthophosphoric acid (40:55:5) at flow rate 1 ml min-1 and wavelength is 265 nm, symmetric peak was obtained.

Linearity:

The correlation coefficient value 0.9996 indicates that the method was linear over a concentration range of 60-210ppm for Sitagliptin phosphate.

Table 1: Data for linear graph

S.NO	Concentration	Peak area
1.	60	288728
2.	90	417581
3.	120	539793
4.	150	684954
5.	180	824260
6.	210	962211

Table 2: Results for regression analysis

S.NO	Drug name	Linear dynamic range (ppm)	Correlation coefficient	Slope	Intercept
1.	Sitagliptin phosphate	60 – 210	0.9996	4548.2	4780.5

Table 3: Results for Accuracy

Level	Target in ppm	Amount of Sitagliptin spiked(ppm)	Total in ppm	Amount of Sitagliptin recovered (ppm)	% Recovery
50%	60	30	90	88.71	98.57
	60	30	90	89.84	99.82
	60	30	90	88.94	98.82
100%	60	60	120	119.57	99.64
	60	60	120	120.62	100.51
	60	60	120	121.02	100.85
150%	60	90	150	152.81	101.87
	60	90	150	151.33	100.89
	60	90	150	152.37	101.58

Table 4: Results for System suitability

S.No	System suitability results	
	USP Plate count	USP Tailing
1	9564	1.28
2	9942	1.34
3	9375	0.56
4	9515	0.66
5	9383	0.78
Mean	9556	0.92

Limit of detection and Limit of quantitation:

The Limit of detection (LOD) and Limit of quantitation (LOQ) represent the concentration of Sitagliptin phosphate stock solution in order to obtain signal-to- noise ratio of 3:1 for LOD and 10:1 for LOQ were determined.

Table 5: Results for LOD & LOQ

Sample	LOD	LOQ
Sitagliptin phosphate	0.05µg/ml	0.16µg/ml

Table 6: Results for Robustness:

Condition	Mean area	% Difference
Unaltered	539793	…….
Flow rate at 0.8ml/min	537519	0.42
Flow rate at 1.2ml/min	538041	0.32
Mobile phase		
MeOH : ACN : 0.1% OP 38:57:05 42:53:05	530367 541617	1.75 034
pH of mobile phase 4.0	538983	0.15
pH of mobile phase 4.2	542948	0.58

Table 7: Results for Intraday precision:

Sample preparation	Observed values	
	Area	% Assay
1	536063	98.8%
2	535151	99.5%
3	530278	97.4%
4	532011	96.8%
5	539975	97.4%
6	526693	99.8%
Mean	533362	98.2%
SD	4691	0.01
%RSD	0.88	1.27

Table 8: Results for Interday precision:

Day	Observed values	
	Area	% Assay
1	531868	97.1%
2	539113	98.4%
3	529707	97.8%
4	526726	99.7%
5	525325	98.9%
6	527193	97.4%
Mean	529989	98.2%
SD	5041	0.01
%RSD	0.95	1.0

Table 9: Quantitative estimation of tablet formulation

Sl.No	Brand name	Standard area	Sample area	Labelled claim (mg)	Amount found (mg)	%Purity
1	Januvia	539793	533644	100	98.50	98.50

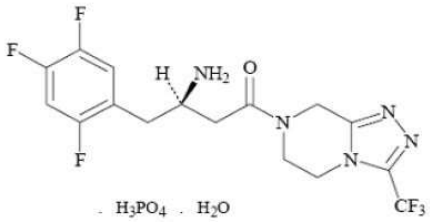

Fig1: Chemical structure of Sitagliptin Phosphate

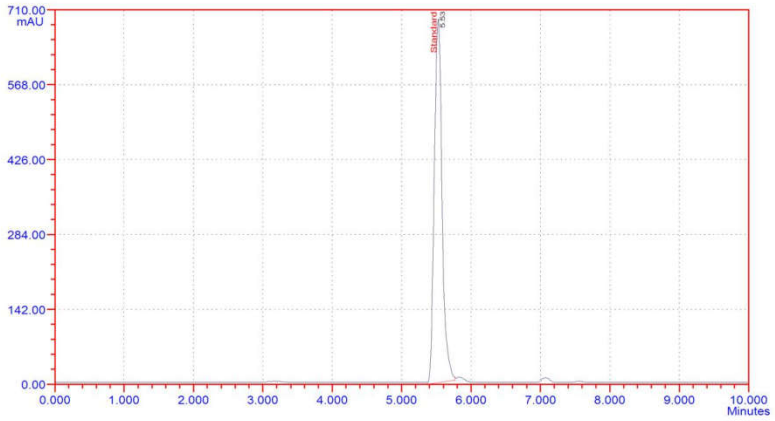

Fig 2: Standard Chromatogram of Sitagliptin Phosphate

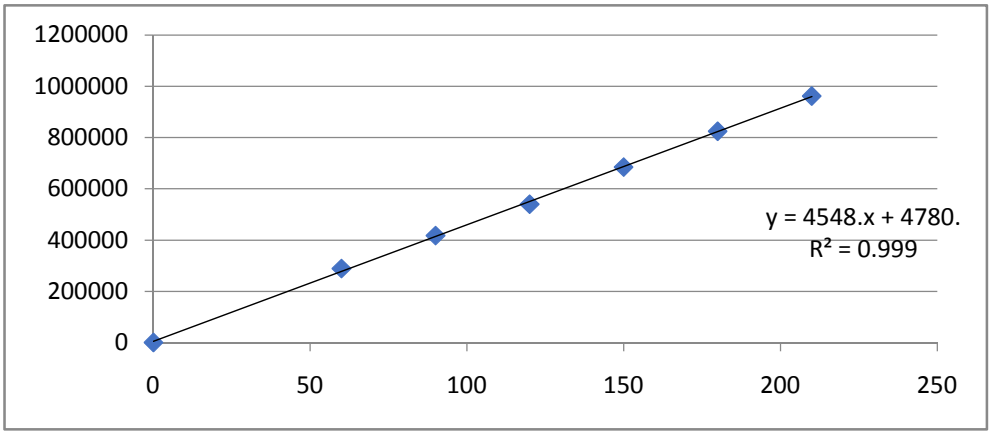

Fig 3: Calibration Curve of Sitagliptin Phosphate

CONCLUSION:

A validated RP-HPLC method has been developed for the quantitative determination of Sitagliptin Phosphate in bulk and pharmaceutical tablet dosage forms. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interference of the other active ingredients and additives used in the

formulation. In fact, results of the study indicates that the developed method was found to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED