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Estimation of Sitagliptin Phosphate monohydrate in presence of its degradants using RP-HPLC

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ABSTRACT

A simple, economic, selective, precise, and stability-indicating HPLC method has been developed and validated for estimation of Sitagliptin both in bulk drug and tablet dosage form. The drug was separated using a mobile phase acetonitrile: water, (5:95 v/v) on an Agilent, TC C_{18} (250 × 4.6 mm) 5µm column at flow rate of 1.0 mL min^{-1} at ambient temperature and detection was performed at 263 nm. The detector linearity was established in concentrations ranging from 10–80 µg mL^{-1}, the regression coefficient was 0.9996. For stability study, the drug was exposed to the stress conditions such as acid, base, oxidation, neutral and sunlight as per the recommendations of ICH guidelines. The results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy. As per ICH guidelines results were found to be satisfactory. The method was proved to be robust with respect to changes in flow rate and temperature. The high recovery and low relative standard deviation confirm the suitability of these methods can be employed for the routine analysis of tablet containing Sitagliptin.

Keywords: Sitagliptin, Acetonitrile, Stability study, ICH, Validation

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INTRODUCTION

Sitagliptin Phosphate Monohydrate, (3*R*)-3-amino-1-[3-(trifluoromethyl)- 5,6-dihydro [1,2,4] triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-onephosphate hydrate (Fig. 1),

is an orally active, potent and selective inhibitor of dipeptidyl peptidase-IV (DPP-IV), for treatment of type 2 diabetes[1-3]. This drug is not official in any Pharmacopoeia. It also improves glycemic control and regulates the growth of insulin producing β-cells in pancreatic islets [4,5]. This is done through inhibition of the inactivation of in cretins, particularly glucagon- like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control [6-8].

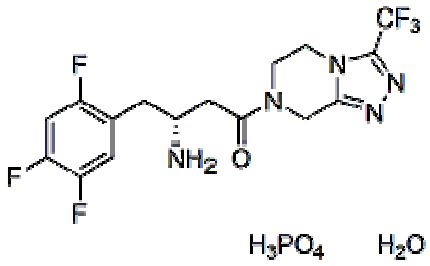


Figure 1: Chemical structure of Sitagliptin Phosphate Monohydrate

Several analytical methods have been reported for the analysis of Sitagliptin such as spectrophotometric [9-11], Spectrofluorimetric [11], RP-HPLC [12,13], LC-MS/MS [14-16], were reported for the determination of sitagliptin phosphate monohydrate in plasma and urine of humans, rats and dogs. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive RP-HPLC method for the estimation of sitagliptin phosphate monohydrate in bulk drug and tablet dosage form. The method was further optimized and validated in accordance with guidelines suggested by International Conference on Harmonization (ICH) [17].

MATERIALS AND METHODS

Authenticate Sitagliptin phosphate monohydrate sample was a kind gift from Matrix Ltd, Sinnar, India. HPLC grade water and acetonitrile (Merck Ltd, Mumbai, India) was used as solvent. All the aqueous reagents were prepared using carbon dioxide free distilled water.

Instrumentation

The HPLC system, Agilent 1120 compact with manual Rheodyne injector facility operates at 20 µL capacity per injection was used. The column was used Agilent TC C_{18} (250 X 4.6 mm) 5µm and the detector consisted of UV/VIS operated at 263 nm. The data were acquired and processed using EZChrom Elite Compact software.

Chromatographic Conditions

Optimizations of chromatographic conditions were carried out using water: acetonitrile (95:5 v/v) as mobile phase. Prior to deliver into the system, mobile phase was filtered through 0.45 µm filter and sonicate for 10 min. The samples were introduced by injector with a 20 µL sample loop. The analysis was carried out under gradient conditions using flow rate 1.0 mL min^{-1} at 18^0C and chromatograms were recorded at 263 nm.

Preparation of standard stock solution:

Weighed accurately 10 mg of sitagliptin phosphate monohydrate and transferred to 100 ml volumetric flask, add 25 mL of mobile phase and sonicate for 15 min and volume was made up to mark with mobile phase (100 µg mL^{-1}).

Preparation of standard solution:

From the standard stock solution 1mL solution was pipetted out in 10 mL volumetric flask and volume was made up to the mark with mobile phase to get a final concentration 10 µg mL^{-1}.

Table1: Assay of Marketed Formulation

Sr. No.	Formulation	Taken Amount	Amount Estimated	% estimated	% RSD
1.		25mg	24.96 mg	99.84	
2.	Januvia	25mg	25.01 mg	100.04	0.1007
3.		25mg	24.99 mg	99.96	
	Mean		24.99 mg	99.95	

RSD is Relative standard deviation

Preparation of Sample Solutions

Twenty tablets of sitagliptin phosphate monohydrate were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 25 mg of sitagliptin (label claim) was transferred to 10 mL volumetric flask, added 5 mL of mobile phase and sonicate for 10 min. The

resultant solution was filtered through 0.45µ membrane filter, diluted to volume with mobile phase. 0.04 mL of resultant solution further diluted to 10 mL and injected to HPLC system (Table 1).

System Suitability:
System suitability parameters were evaluated from retention times, Tailing factor, capacity factor and theoretical plates of standard chromatograms (Table 2).

VALIDATION:
Limit of detection (LOD) and limit of quantification (LOQ):
The signal-to-noise ratio (*S/N*) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the *S/N* ratio and the limit of quantification was estimated as ten times the *S/N* ratio (Table 2).

Specificity:
Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of sitagliptin phosphate monohydrate.

Linearity:
Different standard solutions were prepared by diluting standard stock solution with mobile phase in the concentration range 10-80 µg mL^{-1}. Diluted samples were injected and chromatograms (Fig.2) were taken under standard chromatographic conditions. The peak area was plotted against corresponding concentrations to obtain the calibration graph (Fig.3).

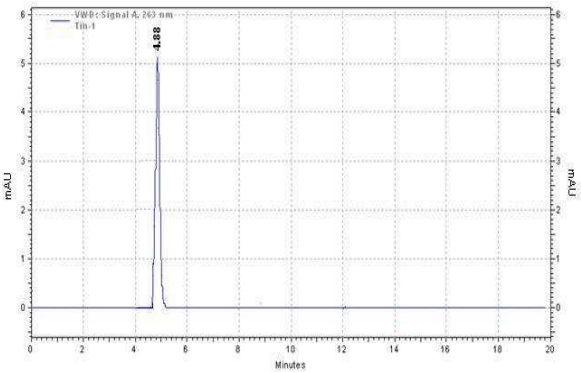


Fig. 2: Chromatogram of Standard Sitagliptin

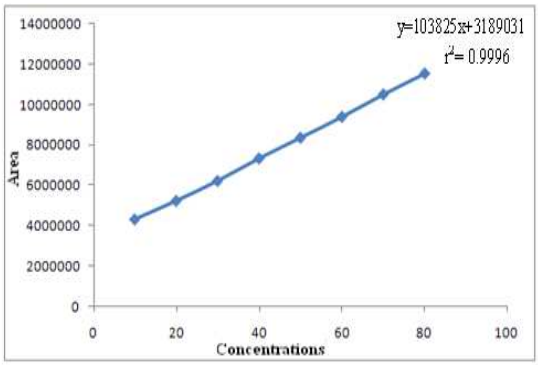


Fig. 3: Calibration plot of Sitagliptin

Table 2: Statistical parameters of Sitagliptin

Parameters	Values
Limit of detection (µg mL^{-1})	0.155
Limit of quantification (µg mL^{-1})	0.469
Linearity (µg mL^{-1})	10-80
Regression equation (y= mx+c)	y=103825x+3189031
Correlation coefficient (r^2)	0.9996
Retention time	4.88
Tailing Factor	1.23
Capacity	2.696
Theoretical Plates	6612

Precision:
Precision of analytical method was expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

Table 3: Precision data of Sitagliptin

Parameters	% estimated	S.D.	%RSD
Intra-day*	100.32	0.315	0.314
	100.94	0.288	0.285
	100.72	0.654	0.650
Inter-day*	101.15	0.980	0.969
	100.50	0.952	0.947
	100.68	0.862	0.856

** indicates mean of three replicates, SD is standard deviation.*

Recovery:

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard sitagliptin corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

Table 4: Recovery study data

Level of standard addition (%)	Amount of tablet powder (mg)	Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery*	% RSD*
80	25	8	8.06	100.75	0.5682
100	25	10	10.12	101.20	0.4941
120	25	12	12.04	100.33	0.4260

Robustness:

Robustness is a measure of the performance of a method when small and deliberate changes are made to the conditions of method. Robustness studies were performed by making slight variations in flow rate and mobile phase composition changes one at a time.

Table 5: Robustness data for Sitagliptin

Parameters	% Recovery	S.D.	% RSD
Change in Mobile Phase Composition(95:5±2)	100.95	0.394	0.390
Change in floe rate (1.0±0.2mL min^{-1})	101.04	0.319	0.316

FORCED DEGRADATION:

Acid and base induced degradation product:

To 10 mL of standard stock solution, 10 ml of 0.1 N HCl and 10 mL of 0.01N NaOH were added separately. These mixtures were reflux separately for 45 min for acid and 10 min for base at 50ºC. The forced degradation study in acidic and basic media was performed in the dark in order to leave out the possible degradative effect of light. 0.1 mL of each resultant solution was diluted to 10 mL with the mobile and resultant solution injected into the system.

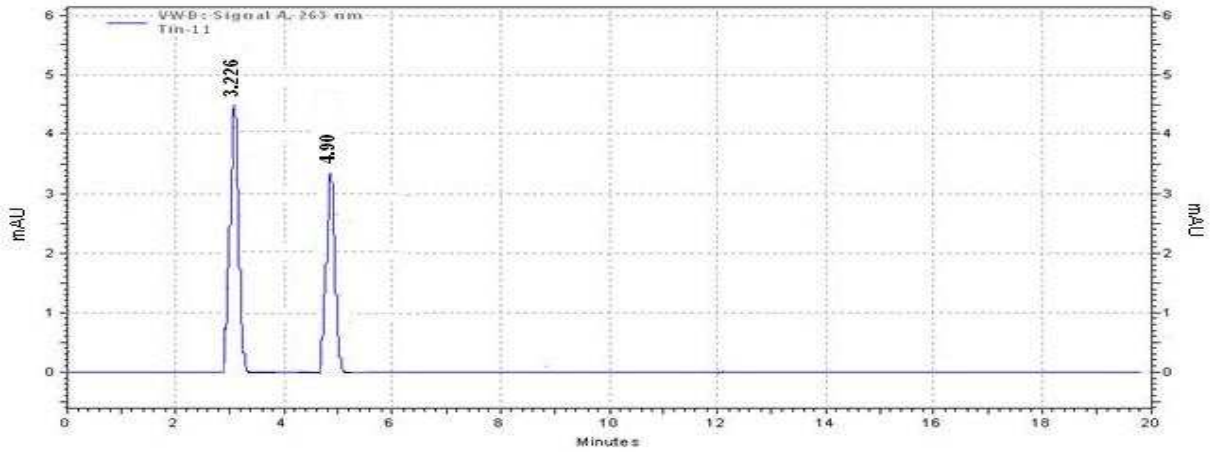


Fig.4: Chromatogram of acid [0.1N HCl (reflux for 45 min at 50°C)] treated sample

Peak 1, degradant [Rt = 3.226]; Peak 2, Sitagliptin [Rt=4.90]

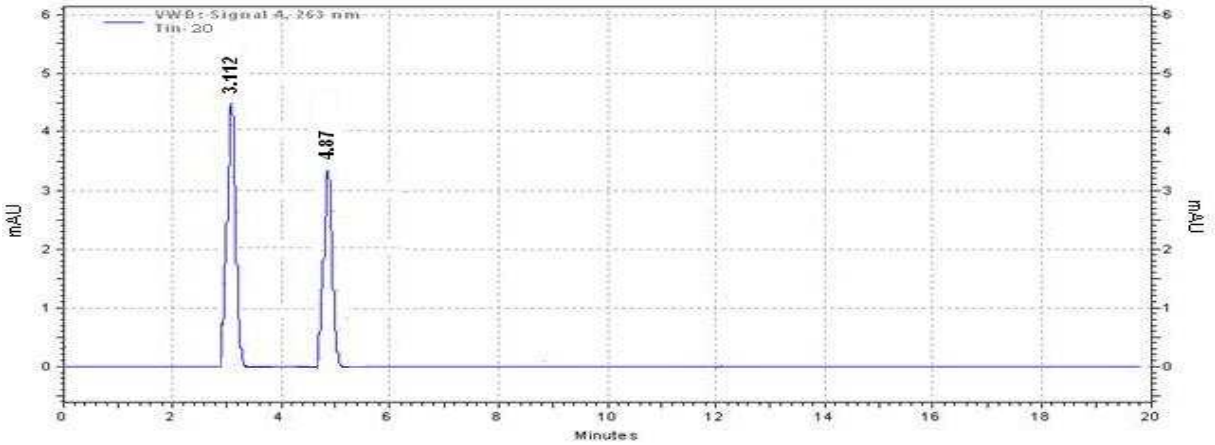


Fig.5: Chromatogram of base [0.01N NaOH (reflux for 10 min at 50°C)] treated sample
Peak 1, degradant [Rt = 3.112]; Peak 2, Sitagliptin [Rt=4.87]

Hydrogen peroxide induced degradation product:
To 10 mL of standard stock solution, 10 mL of hydrogen peroxide (H_2O_2) (3 % v/v) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and reflux for 20 min at 50°C. 0.1 ml of resultant solution was diluted to 10 mL with the mobile phase and resultant solution injected into the system.

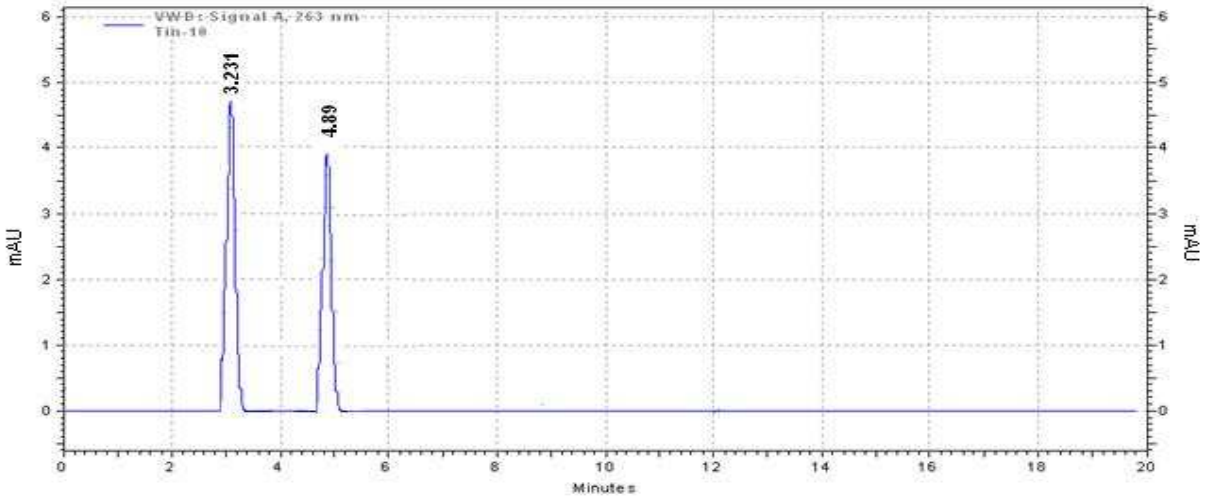
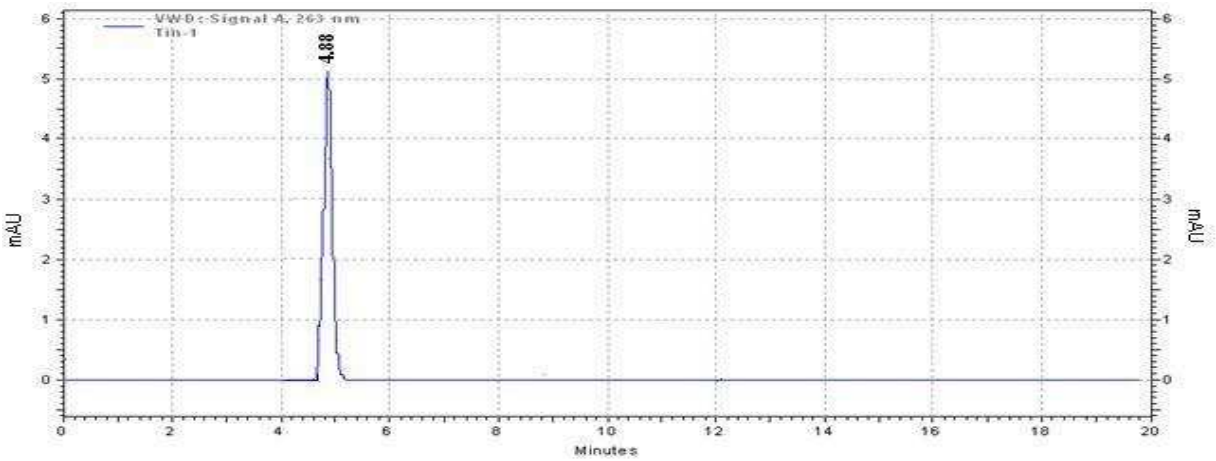


Fig.6 Chromatogram of Hydrogen peroxide [3% H_2O_2 (reflux for 20 min at 50°C)] treated sample
Peak 1, degradant [Rt = 3.231]; Peak 2, Sitagliptin [Rt=4.89]

Neutral hydrolysis:
10 mL of standard stock solution was mixed with 10 mL water and reflux for 60 min at 60ºC. 0.1 ml solution this solution was diluted to 10 mL with the mobile and resultant solution injected into the system.



FiFig.7 Chromatogram of neutral hydrolysis (reflux for 60 min at 60°C)]
Peak 1, Sitagliptin [Rt = 4.88]

Photolytic induced degradation product: 10 mL of standard stock solution was exposed to direct sunlight for 30 min on a wooden plank and kept on terrace. 0.01 ml of resultant exposed solution was transferred to 10 mL volumetric flask, diluted with the mobile phase and solution was injected into the system.

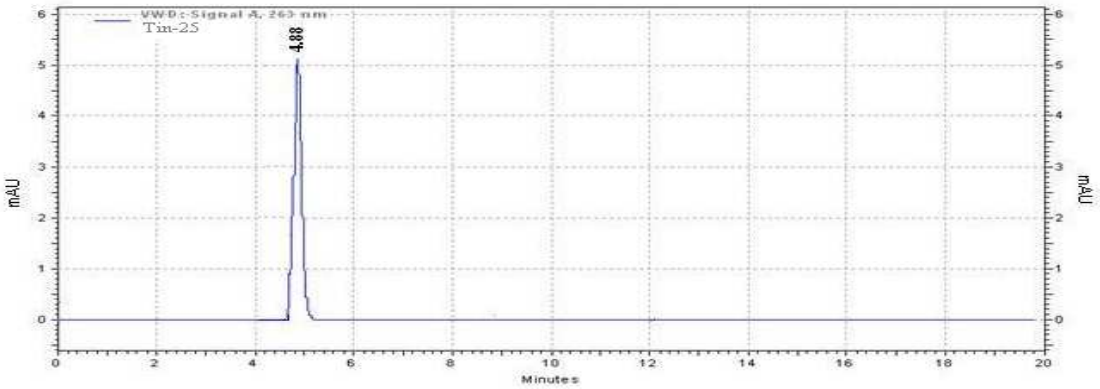


Fig.8 Chromatogram of sunlight exposed (for 30 min) sample
Peak 1, Sitagliptin [Rt = 4.88]

RESULTS AND DISCUSSION

The parameters were focused for improvisation of retention time, separation of degradation products and column life. The Agilent TC C_{18} column provided good peak shapes and no peak splitting was observed. Sitagliptin showed linear responses in concentrations level ranging from 10-80 µg mL^{-1} with correlation co-efficient 0.9996 (Table 2).

The measurement at three different concentration levels showed low value of % R.S.D. (< 2) and low value of S. E. (< 2) for intra- and inter-day variation, which suggested an excellent precision of the method.

The recovery of drug was determined by spiking drug at three different levels and was found to be between 100.33 - 101.20. The method was found to be robust with respect to flow rate and change in mobile phase composition without any changes in system suitability parameters.

Forced degradation of drug was carried out as per the ICH guidelines (ICH Q2B) by subjecting sitagliptin to various stress conditions. The percent area decreased at the level of 11-26 % and additional peaks at retention time different to that of well separated peak of sitagliptin indicated that sitagliptin undergoes degradation in acidic, basic and oxidative conditions. Summary of force degradation data are given in Table 6.

Table 6: Summary of force degradation data

Sample stress condition	Stress condition	Sitagliptin (R.T.)	Degradants (R.T.)	% Area decreased	Fig. No
Acid degradation	0.1 N HCl reflux for 45 min.	4.90	3.226	25.40	4
Alkaline degradation	0.01 N NaOH reflux for 10 min	4.87	3.112	21.54	5
Oxidative degradation	6% H_2O_2 reflux for 20 min.	4.89	3.231	10.50	6
Neutral hydrolysis	Purified water reflux for 1 hr.	4.88	--	--	7
Photolytic degradation	Kept in sunlight for 30 min.	4.88	--	--	8

CONCLUSION

The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. As the method able to separate the parent drug from degradation products it can be employed as a stability indicating method for sitagliptin.

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