

Original Article

Analytical method development and validation for some oral hypoglycemic drugs

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Abstract

A rapid, precise, accurate, specific and simple RP-HPLC analytical method were developed for the estimation of Sitagliptin (SITA) in tablet formulation as well.

A new reverse phase HPLC method was developed for the estimation of Sitagliptin phosphate in bulk and dosage forms. Chromatography was performed by gradient reverse phase separation using a C₁₈ column of particle size 5microns. Mobile phase used was Potassium dihydrogen phosphate buffer of 3.0 pH and Acetonitrile in the ratio of 35:65. The flow rate was 1ml/min and the effluent monitored at 210 nm. The retention time was observed at 2.5 for Sitagliptin phosphate. The standard curve was linear over a working range of 5-30µg/ml and gave an average correlation factor of 0.997. The limit of detection and the limit of quantitation were found to be 0.087 and 0.826 respectively. The method showed good recoveries (98%-102%) and relative standard deviations of intra and inter day assay less than 2. This method can be easily and conveniently used for routine analysis of Sitagliptin (sita) phosphate.

Key Words: Sitagliptin; RP-HPLC; Acetonitrile; Potassium dihydrogen phosphate.

Introduction

Sitagliptin¹ is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor² which improves glycemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). This increases active incretin and insulin levels, and decreases glucagon levels and post-glucose-load glucose excursion. Progressive β -cell dysfunction and β -cell failure are fundamental pathogenic consequences of type 2 diabetes³. Dipeptidyl peptidase-4 inhibitors may exhibit improvement on preclinical measures of β -cell function.

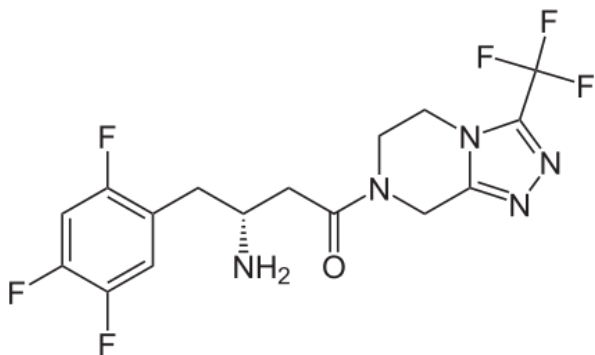


Fig.1.1 Structure of sitagliptin phosphate

Literature survey reveals that, various analytical strategies have been used for the measurement of Sitagliptin in combination with various drugs in plasma and pharmaceutical preparations.^{4, 5} Few analytical methods that have been reported for the determination of Sitagliptin in Pharmaceutical formulations include RP-HPLC^{6,7,8} and UV Spectrophotometry^{9,10} individually, but the present developed method was cheaper, time saving and effective than the previous methods.

Materials and Methods:

Chromatographic conditions:

The analysis of the drug was carried out on a gradient high pressure liquid chromatography (Agilent) LC COMPACT – 1120 equipped with a reverse phase XBridge C-18 Column (250 x 4.6 mm i.d, particle size 5 μ m), a binary pump, a 20 μ l rheodyne injection loop and a variable UV detector running on EZ CHROME ELITE (Agilent) software.

Chemicals and solvents:

Analytically pure sample of Sitagliptin phosphate procured as gift sample by Bioplus Life Sciences, Bengaluru. The drug is used without further purification. HPLC grade Acetonitrile (Merck), Pharmaceutical formulation. Tablets (label claim 80mg SITA) were used in HPLC analysis. HPLC grade water obtained in-house by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study. Analytical grade reagents Potassium Dihydrogen Phosphate (Merck) and HPLC grade o-phosphoric acid (Merck) were used in the estimation of Sitagliptin by HPLC study.

Preparation of Potassium dihydrogen phosphate buffer p^H 3.0:

0.68g of Potassium dihydrogen phosphate was accurately weighed and transferred into 500ml volumetric flask and dissolved in water and pH was adjusted to 3 using ortho phosphoric acid.

This solution was filtered through a 0.45 μ m nylon membrane (PALL) and degassed by ultra sonicator.

Preparation of mobile phase:

Transferred 1000ml of above solution and 1000ml of Acetonitrile to the mobile phase bottles separately. HPLC experiments were carried out using binary pump A containing Acetonitrile and pump B containing Potassium dihydrogen phosphate buffer in the ratio of 65:35.

Preparation of standard stock:

25mg of Sitagliptin phosphate standard was accurately weighed and transferred to a 25ml volumetric flask, dissolved in 10ml of methanol and sonicate for 10mins and make up with Acetonitrile to give a solution containing 1000 μ g/ml. (stock solution 'A') From this stock solution, pipetted out 5ml, placed in to 50ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100 μ g/ml. (stock solution 'B'). From the above 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0ml of solutions were pipetted out into separate 10ml volumetric flasks and volume was made up to the mark with the Acetonitrile. This gave the concentration of 5, 10, 15, 20, 25, and 30 μ g/ml. These six dilutions of Sitagliptin phosphate were prepared and are estimated in HPLC.

Preparation of sample solution:

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 80mg of was transferred to 100ml volumetric flask containing 15ml of methanol and the contents of the flask were sonicate for 10min, to ensure the complete solubility of the drug, then the mixture was made up to 100ml with Acetonitrile.

The resulting solution was thoroughly mixed and filtered through a 0.45 μ m membrane filter. From this solution, required dilutions for HPLC method were prepared within the linearity range using diluents as solvent.

Calibration curve:

The calibration curve was plotted with six concentrations of the standard drug solution 5-30 μ g/ml and chromatography was repeated six times for each dilution. The linearity was evaluated by linear regression analysis. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Six determinations were carried out for each solution, peak areas were recorded for all the solutions. All stock and working solutions were sonicated for 10 min then filtered through the nylon membrane filter (0.45 μ) prior to use. 20 μ L injections were made for each concentration and chromatographed six times under specified condition at ambient temperature (25^oC). The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection v/s the

injected amounts of the respective concentrations. Fig no 3.3 show the retention time of the drug.

Precision, accuracy and selectivity:

Precision, accuracy and selectivity were studied systematically as per the ICH guidelines for the estimation of Sitagliptin phosphate. Solution containing 5 µg/ml of Sitagliptin was subjected to the proposed HPLC analysis to check method precision and intermediate precision. The accuracy of the HPLC method was assessed by analysing the solutions of Sitagliptin at 50, 100 and 150 % concentration levels by the proposed method. It was evaluated by back calculation. The specificity of the method was evaluated with regard to interference due to presence of any other excipients. It shows that drug was clearly separated from its excipients. Thus, the HPLC method presented in this study is selective.

LOD & LOQ:

LOD & LOQ was calculated using standard deviation as per the ICH guidelines.

LOD = 3.3 * standard deviation / slope

LOQ = 10 * standard deviation / slope

Results & Discussion

System suitability was performed to verify the working condition of chromatographic system to get accurate and precise results by using standard solution. The RSD for area response obtained from six replicate injections was found to be within the limits, the tailing factor was found to be 1.09 which was well within the acceptance criteria of NMT 2.0 and NMT 2.0 respectively. The number of theoretical plates is a measure of column efficiency which shows the high separation efficiency of the column used and was found to be 9732 for Sitagliptin which is in the acceptance criteria of NLT 2000. It was observed from the results that the system suitability parameters meet the requirement of method validation.

Specificity of the method was determined and the peaks of diluent, mobile phase and excipients of tablets did not interfere with the peak of Sitagliptin (fig. no. 3.1).

The method was found to be linear over a concentration range of 5 to 30 µg/ml the correlation coefficient was found to be 1 within the acceptance criteria limit of NLT 0.995. the results are given in the table no. 3.1.

Precision was carried out on same day as well as on different days to ensure that analytical results remain unaffected with change in day. The RSD was calculated for standard and sample on six determinations and was found to be less than 2.0 (table no. 3.4).

Accuracy of the method was performed to ensure closeness of agreement between true value and

reference value in three levels each. The mean % recoveries at concentrations ranging from 50%, 100% and 150% were found to be 100.81%, 99.74%, 99.37% (table no. 3.3) which were in the acceptance limit of 98.0 to 102%.

The results of LOD & LOQ were given in the table no. 3.2.

Table: 3.1 Linearity data for Sitagliptin Phosphate

S.No	Sitagliptin phosphate	
	Concentrations µg/ml	Peak area
1	5	4021853
2	10	7532808
3	15	12320166
4	20	15643239
5	25	20315210
6	30	23588079

Fig No: 3.1 Linearity curve of Sitagliptin Phosphate

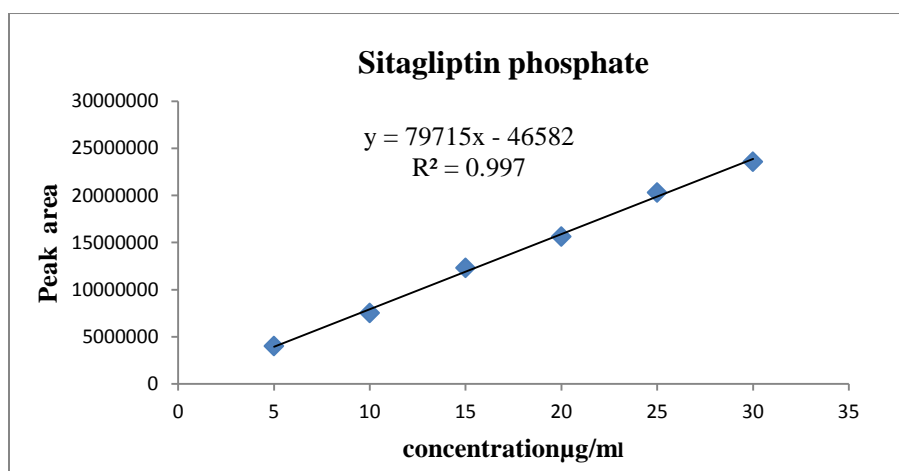


Table: 3. 2 LOD & LOQ of Sitagliptin phosphate

Parameters	$\mu\text{g/ml}$
LOD	0.087
LOQ	0.289

Fig no. 3.2 Chromatogram of blank

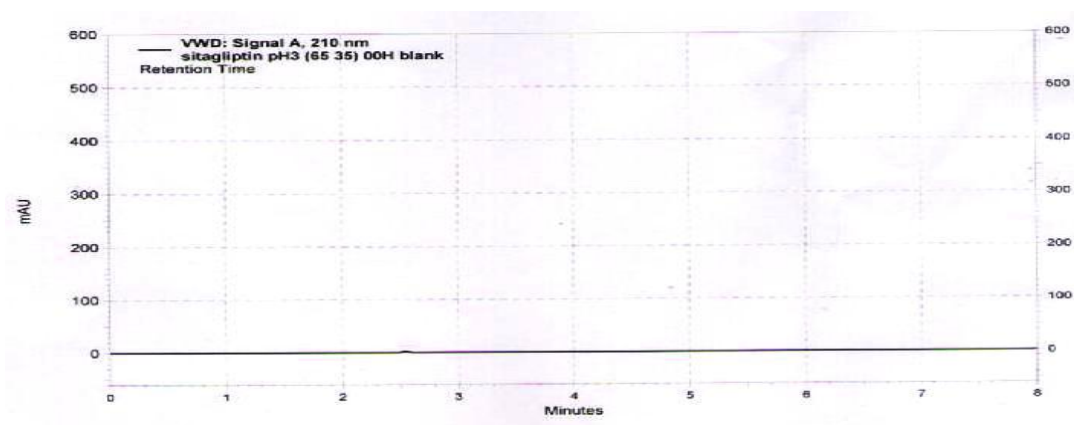


Fig no. 3.3 Chromatogram showing the retention time of Sitagliptin

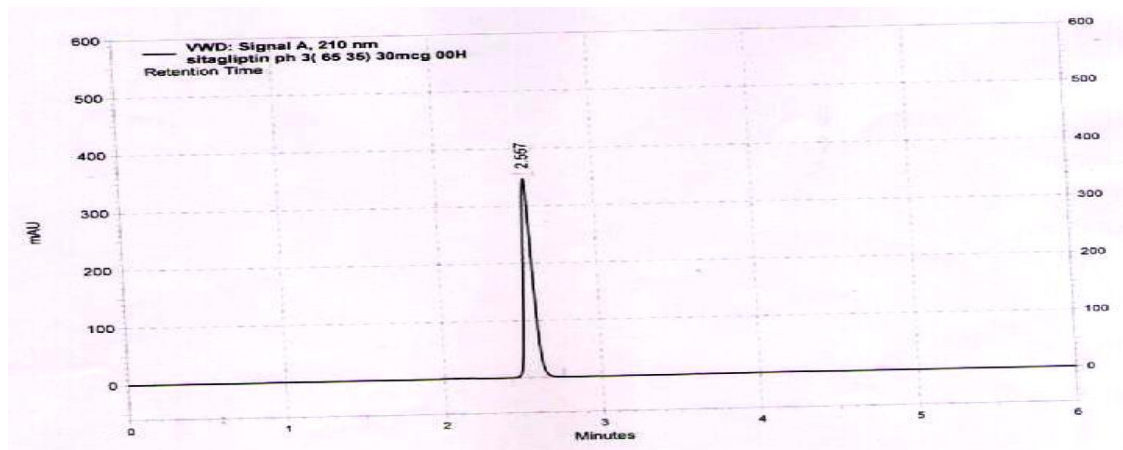


Table: 3.3 Assay for pharmaceutical formulations

S.No	Labeled Amount	RP-HPLC		
		Amount added	Amount Found	%Recovery
1	80	40	10.0853	100.853
2	80	80	10.1284	101.284
3	80	120	10.1361	101.361

**Mean of three determinations

Table no 3.4 Precision study results by HPLC

S.No	DAY1		DAY2	
	INJECTION	AREA	INJECTION	AREA
1	Injection1	7463264	Injection1	7593594
2	Injection2	7586946	Injection2	7631877
3	Injection3	7565241	Injection3	7540842
4	Injection4	7631572	Injection4	7465806
5	Injection5	7546133	Injection5	7523846
6	Injection6	7489143	Injection6	7584624
	Average	7547050	Average	7596798
	SD	62355	SD	105811.2
	%RSD	0.826	%RSD	1.392

Conclusions

The Proposed RP-HPLC is a suitable technique for determination of Sitagliptin . All the parameters for analysing Sitagliptin met the criteria of ICH guidelines for method validation. The developed method may be recommended for routine QC analysis of the investigated drugs to provide simple and accurate quantitative analysis for the determination of Sitagliptin.

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