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Development and Validation of an RP-HPLC Method for Analysis of Sitagliptin

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ABSTRACT

Sitagliptin is a drug used against type 2 diabetes mellitus and it is a member of class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins". A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Sitagliptin in bulk formulation. The λmax of the Sitagliptin was found to be 267nm in Methanol: phosphate buffer 10mM pH 4.8 [60:40(v/v)]. The method shows high sensitivity with linearity 10 to 50µg/ml (regression equation: y = 45765x + 239272; r^2 = 0.9996).

The various parameters according to ICH guidelines and USP are followed for validating and testing of this method. The Detection limit and quantitation limit were found to be 0.743 µg ml^{-1} and 2.25µgml^{-1} respectively. The results demonstrated that the procedure is accurate, specific and reproducible (RSD < 2%), and also being simple, cheap and less time consuming and appropriate for the determination of Sitagliptin in bulk and pharmaceutical formulation.

KEYWORDS: *Sitagliptin, dipeptidyl peptidase-4 inhibitors, HPLC method.*

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INTRODUCTION

This Anti-diabetic drug shows the action by decreasing the effects of a protein or enzyme (by the inhibition of this protein or enzyme) on the pancreas at the level of release of glucagon (diminishes its release) and at the level of insulin (increases its synthesis and release) until glucose level in blood is restored toward normal, and this happens in the case of, protein/enzyme-enzyme inhibitor becomes less effective and the amounts of insulin released diminishes thus diminishing the "overshoot" of hypoglycemia seen in other oral hypoglycaemic agents. The drug is very specific. Dipeptidyl peptidase-4 inhibitor, which shows its actions in type 2 diabetic patients by slowing the inactivation of incretin hormones, thereby increasing the prolonging action and concentration of these hormones.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP) are the Incretin hormones which are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly terminated by the enzyme, Dipeptidyl peptidase-4 (DPP-4). GLP-1 and GIP increase insulin synthesis when the blood glucose concentrations are normal or elevated, and are release from pancreatic beta cells by intracellular signalling pathways involving cyclic AMP.

The mode of action of Sitagliptin is by competitively inhibiting a protein/enzyme i.e. dipeptidyl peptidase-4 (DPP-4), which results in an increased amount of active incretins (GLP-1 and GIP), and reduces amount of release of glucagon (diminishes its release) and also increased release of insulin. Glucagon-like peptide-1 also lowers glucagon secretion from alpha cells (in pancreas), which ultimately leading to reduced hepatic glucose production.

There are few adverse effects of Sitagliptin are very similar to placebo, like rare nausea, common cold-symptoms, and photosensitivity. Sitagliptin is recommended by medical practitioner as a second-line drug (single or in combination with other drugs) after the combination of diet/exercise and metformin fails.

The patients with moderate-to-severe renal impairment; A dose of 25-50 mg once daily is recommended. With oral administration Sitagliptin has bioavailability of 87% and the elimination of sitagliptin occurs primarily via renal excretion and involves active tubular secretion.

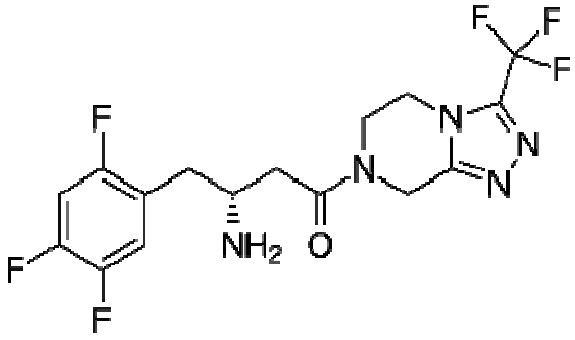

Fig-1: Structure of Sitagliptin

MATERIALS AND METHOD:

Instruments:

The chromatographic separation was performed on Analytical Technologies HPLC-3000 series compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used. Model - UV 2012 double beam UV visible spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic balance was used for Spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Sitagliptin sample was procured from Swaroop Drugs and Pharmaceuticals, Aurangabad. HPLC grade Methanol and HPLC grade Water were procured from Merck specialities private limited, Mumbai.

Chromatographic conditions

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used for the chromatographic separation at a detection wave length of 246 nm. Methanol, Phosphate buffer pH 4.8 in a ratio of 60:40 v/v was selected as mobile phase for elution and same mixture was used in the preparation of standard and sample solutions. The elution was monitored by injecting the 20µl and the flow rate was adjusted to 0.8 ml/min.

Preparation of Mobile phase

Preparation of Phosphate buffer pH 4.8: Dissolved 1.36g of Potassium dihydrogen orthophosphate & 2ml of triethylamine in 800ml of HPLC water, adjusted the pH to 4.8 with orthophosphoric acid and added sufficient HPLC water to produce 1000ml.The mobile phase was sonicated for 15 min and filtered through a 0.45 µm membrane filter paper.

Preparation of Standard solutions

10mg Sitagliptin was accurately weighed and transferred into 10 ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solution of concentration 1000µg/ml of the drug. (Working stock solution).

Preparation of Sample Solution

20 tablets of Sitagliptin were initially weighed and powdered and an amount equivalent to 10mg was accurately weighed into a 10ml volumetric flask, mixed with 10ml of mobile phase and sonicated for 5 min after making final volume up to 10 ml with mobile phase. Then solution was filtered through 0.45µm membrane filter. The solution contains 1000µg/ml of Sitagliptin. From the above stock solution, 0.1ml aliquot was transferred in to a 10 ml volumetric flask, volume was made up to the mark with mobile phase to obtain a final concentration of 10 µg/ml of metformin.

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a estimation of Sitagliptin. Different mobile phases were tried for the method optimization, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, Phosphate buffer pH 4.8 (60:40 v/v) using C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] Table:1 and a typical chromatograph of Sitagliptin was shown in figure 3.

Parameter	Condition
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	60 : 40 (Methanol: Phosphate buffer pH-4.8).
Flow Rate	0.8 ml/min
Wavelength	267 nm
Injection Volume	20 µl
Detector	UV-3000-M
Run Time	7.5 min
Retention Time	Approx. 4.6 min

Table 1: Optimized parameter

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (R) guidelines.

1. Linearity

For the determination of linearity, appropriate aliquots were pipetted out from 1000µg/ml (working stock solution). 0.1 – 0.5 ml was pipetted out in to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of metformin. Each solution was injected in triplicate. Calibration curves were plotted with concentration against observed peak areas followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curve for Sitagliptin was shown in figure 2 and their corresponding linearity parameters given in table 2.

2. Accuracy

To ensure the reliability and accuracy of the recovery studies were carried out by % recovery method (standard addition method). A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the per cent recovery was reported. The results were given in table 4.

3. Precision

The repeatability of the method was verified by calculating the % RSD of three replicate injections of 100% concentration (30µg/ml of Sitagliptin) on the same day and for intraday precision % RSD was calculated from repeated studies. The results were given in table 5.

4. Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 s/s and LOQ = 10 s/s.

5. Robustness

Robustness was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported. In the operational conditions Small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wave length and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate. % RSD was reported in the table 6.

6. Assay of marketed formulation

20 tablets of Sitagliptin were weighed and crushed into fine powder. The average weight of the tablet was calculated and the amount equivalent to 10 mg of pure Sitagliptin was dissolved in 10 ml of solvent. From this stock solution 30 ppm dilution was prepared and injected. The % purity was calculated by comparing the result with result obtained from 30 ppm standard drug and are reported in table 7.

7. System suitability

It was ensured that from the system suitability parameters, the method can generate results of acceptable accuracy and precision. System suitability was carried out with three injections of solution of 30 µl/ml of Sitagliptin in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table 8.

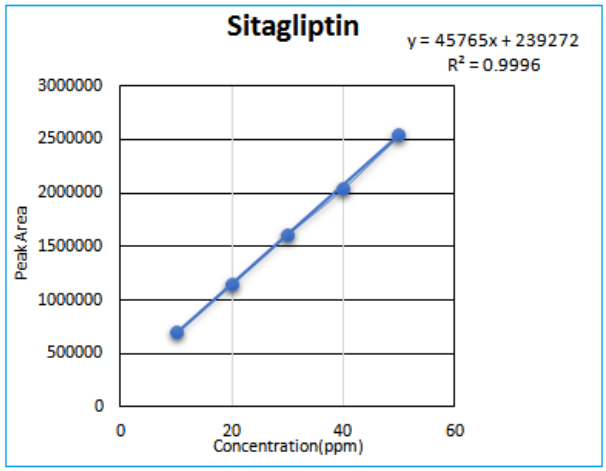

Figure 2: Linearity

RESULT AND DISCUSSION

Linearity:

It was clarified from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figure 1) gave linear relationship over the concentration range of 10 - 50 µg/ml for Sitagliptin. From the regression analysis, a linear equation was obtained y = 45765x + 239272, and the goodness-of-fit (r^2) was found to be 0.9996, indicating a linear relationship between the concentration of analyte and area under the peak.

Conc. (µg/ml)	Peak Area
10	701859
20	1153624
30	1615753
40	2045842
50	2543985

Table 2: Summary of results of Linearity

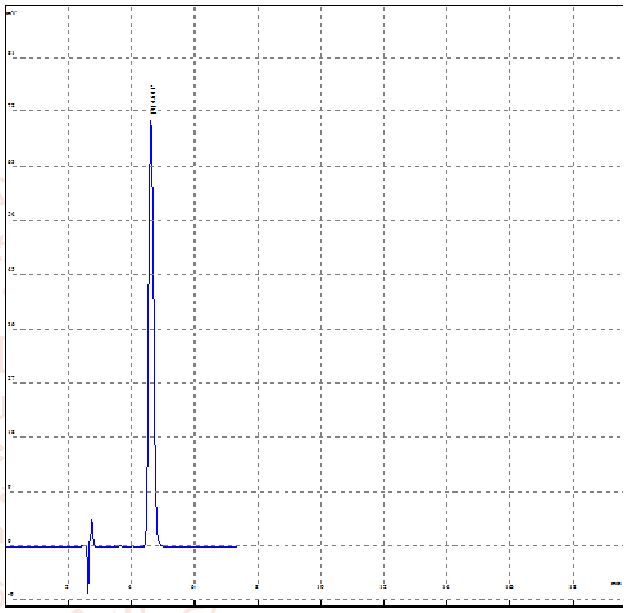

Figure 3: Typical chromatograph of Sitagliptin

Accuracy

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Sitagliptin and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as demonstrated in Table -3.

Table 3: summary of Results of Accuracy

Sr. No.	Conc.(ppm)	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10	703954	701792.6667	2195.25177	0.3128063	0.312806314
	10	701859				
	10	699565				
2	30	1603548	1617282	14558.84209	0.9002043	0.900204299
	30	1615753				
	30	1632545				
3	50	2553154	2540818	14187.13844	0.5583689	0.558368936
	50	2543985				
	50	2525315				

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	1615753	1619565	100.24
2	100% Recovery	2045842	2043025	99.86
3	150% Recovery	2543985	2562546	100.73

Table 4: % recovery

Precision

Precision is "the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is expressed in the form of relative standard deviation. The repeatability, intra-day and inter-day precision results are shown in the table 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD as demonstrated in Table 5.

Interday	**Day 1**			**Day 2**		**Mean**	**%RSD**
1621668	1615753	1620656	1624652	1617288	1610635	1610635	**0.31%**
Intraday	**Morning**			**Evening**			**%RSD**
1621668	1611325	1615753	1614155	1620656	1623956	1617919	0.30%

Table-5: summary of Precision

LOD and LOQ

The LOD and LOQ were calculated by the equations LOD $= \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$ and LOQ $= \frac{10 \times \text{std.Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Sitagliptin were 0.743 and 2.25 µg/ml, respectively.

Robustness

Robustness of the method reflects that the results are unaffected or reliable even if the minute changes in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 30µg/mL solution are as shown in Table No. 6.

Parameter	conditions	Conc.(ppm)	Area	Mean	SD	%SD
Flow rate(ml)	0.7	30	1612589	1615535	2843.75	0.1760
	0.8	30	1615753			
	0.9	30	1618264			
Wavelength (nm)	265	30	1606531	1606150	9799.57	0.6101278
	267	30	1615753			
	269	30	1596165			

Table 6: Robustness

Assay of marketed formulation

The % purity obtained from the formulation was given in table 7. And it was found that the Assay results of Sitagliptin are within the limits.

Sr. No.	% Composition	Area of standard	Area of sample	%Assay
1	%Assay	1615753	1586341	98.1797

Table 7: Assay of tables of Sitagliptin

System Suitability Parameters:

System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plate, asymmetry factor are satisfactory. The chromatographs confirm the presence of Sitagliptin at 4.6 min without any interference.

Parameter	Observed Value	Limits
No. of Theoretical Plates	9650	> 2000

Table 8: System suitability parameter

CONCLUSION:

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Sitagliptin from pure and its dosage forms. The mobile phase used for method development is very simple to prepare and economical also. The sample recoveries in the formulation were showing good results with their respective label claims and it was found that there is no interference of formulation excipients in the estimation. And hence, this method can be easily and conveniently adopted for routine analysis of Sitagliptin in pure form and its dosage form.

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