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Development and Validation of an HPLC Method for the Analysis of Saxagliptin in Bulk Powder

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ABSTRACT

The aim of this study was to develop a simple, rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method for quantification of Saxagliptin in pharmaceutical bulk powder.

The chromatographic system employs isocratic elution using a Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron). Mobile phase consisting of Methanol and water (70:30) set at flow rate 0.8 ml/min. The analyte was detected and quantified at 212nm using ultraviolet detector. The method was validated as per ICH guidelines.

The standard curve was found to have a linear relationship (r2 > 0.99) over the analytical range of 10-50 µg/ml. For all quality control (QC) standards in intraday and interday assay, demonstrating the precision and accuracy over the analytical range. Samples were stable during preparation and analysis procedure.

Therefore the rapid and sensitive developed method can be used for the routine analysis of Saxagliptin such as dissolution and stability assays of pre- and post-marketed dosage forms.

KEYWORDS: *Saxagliptin, dipeptidyl peptidase-4 inhibitors, Diabetes mellitus*

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INRODUCTION

Saxagliptin is pharmaceutical drug of class of type-2 Diabetes which is Dipeptidyl peptide-4 and shows mechanism of action for the same. It has very fewer systemic side effect because of it is very specific to DPP-4 inhibition.

Saxagliptin shows inhibition of enzymatic activity of dipeptidyl peptidase-4 for a period of 24-hours. The working of class of DPP-4 inhibitors is by affecting the action of natural hormones in the body called incretins, which decreases blood sugar from increasing consumption of sugar by the body and reducing production of sugar by the liver, mainly through increasing insulin production in the pancreas.

Mechanisms of action of Dipeptidyl peptide-4 is by two ways, an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated. Saxagliptin forms a reversible, histidine-assisted covalent bond between its S630 hydroxyl oxygen and nitrile group on DPP-4 enzyme.

The levels of active glucagon like peptide 1 (GLP-1) is increases by the inhibition of Dipeptidyl peptidase-4, which inhibits glucagon production from pancreatic alpha cells and increases production of insulin from pancreatic beta cells. It is a well-tolerated agent with very fewer adverse effects which include infection to upper respiratory tract, urinary tract infection, and headache. And that Hypoglycemia, weight gain, and adverse cardiovascular events are negligible as compared with other oral Anti-Diabetic drugs. In clinical studies, Saxagliptin was found to be with high efficacy and well tolerated when used as a monotherapy as well as in combination with Saxagliptin.

Saxagliptin is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl)acetyl]-2-azabicyclo [3.1.0]hexane-3-carbonitrile ($C_{18}H_{25}N_3O_2$), and structure showing in Fig: 1.

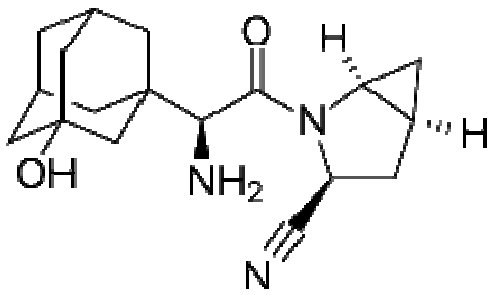

Fig-1: Saxagliptin

In the patients having severe renal impairment, the dose reduction is required. The bioavailability of Saxagliptin was 67% because it is rapidly absorb by orally. Saxagliptin is excreted by both renal and hepatic pathways. 75% of Saxagliptin is eliminated in the urine and 22% in the faeces.

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 Saxagliptin 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 212 nm. Methanol, water in a ratio of 70:30 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 Standard solutions

10 mg Saxagliptin 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 Saxagliptin 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 Saxagliptin. 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 Saxagliptin.

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a estimation of Saxagliptin. Different mobile phases were tried for the method optimization, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, water (70:30 v/v) using C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] Table:1 and a typical chromatograph of Saxagliptin was shown in figure 3.

Parameter	Condition
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	70 : 30 (Methanol: Phosphate buffer pH-4.8).
Flow Rate	0.8 ml/min
Wavelength	212 nm
Injection Volume	20 µl
Detector	UV-3000-M
Run Time	7.5 min
Retention Time	Approx. 000 min

Table1: 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 Saxagliptin. 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 Saxagliptin 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 Saxagliptin) 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 Saxagliptin were weighed and crushed into fine powder. The average weight of the tablet was calculated and the amount equivalent to 10 mg of pure Saxagliptin 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 ensure 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 Saxagliptin in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table 8.

RESULT AND DISCUSSION

Linearity*:*

It was clarify 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 Saxagliptin. From the regression analysis, a linear equation was obtained y = 39629x + 44646, and the goodness-of-fit (r^2) was found to be 0.9989, indicating a linear relationship between the concentration of analyte and area under the peak.

Conc. (µg/ml)	Peak Area
10	437246
20	863287
30	1205362
40	1622726
50	2038985

Table2: Summary of results of Linearity

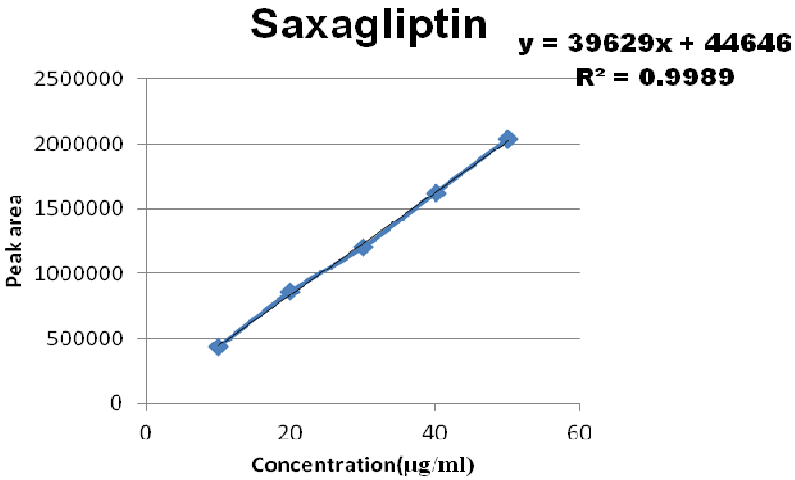

Figure2: Linearity

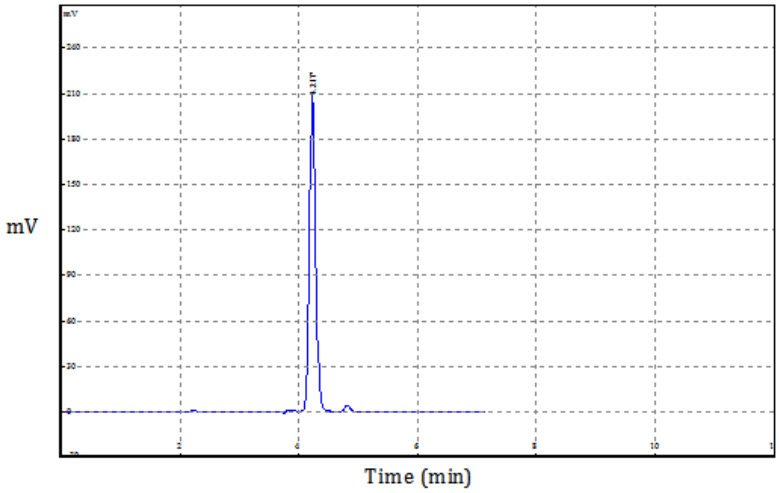

Figure 3: Typical chromatograph of Saxagliptin

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 Saxagliptin 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.

			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.(ppm)	Area	Mean	SD	%SD	%RSD
	10	437589				
1	10	437246	437430.3333	172.934477	0.0395342	0.039534176
	10	437456				
	30	1208494				
2	30	1205362	1207139.333	1608.2106	0.1332249	0.133224936
	30	1632545				
	50	2036434				
3	50	2038985	2038528.333	1907.449694	0.0935699	0.093569938
	50	2040166				

Table3: summary of Results of Accuracy

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	1205362	1208921	100.295264
2	100% Recovery	1622726	1639656	101.0433061
3	150% Recovery	2038985	2034615	99.78567768

Table4: % 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
1197654	1205362	1206495	1208656	1201659	1203564	1203564	**0.32%**

Intraday	Morning		Evening				%RSD
1205362	1219645	1206659	1197654	1196523	1206495	1205390	**0.69%**

Table-5: summary of Precision

LOD and LOQ

The LOD and LOQ were calculated by the equations LOD $\frac{3.3\times std.Deviation}{slope}$ and LOQ = $\frac{10\times std.Deviation}{slope}$ where, std. Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Saxagliptin were 0.102 and 0.3101 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	1207634			
	0.8	30	1205362	1205876	1565.61	0.12983211
	0.9	30	1204632			
Wavelength (nm)	210	30	1201649			
	212	30	1205362	1203557	1858.61	0.1544268
	214	30	1203659			

Table6: 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 Saxagliptin are within the limits.

Sr. No.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	1205362	1191981	98.8899

Table7: Assay of tables of Saxagliptin

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 Saxagliptin at 4.2 min without any interference.

Parameter	Observed Value	limits
No. of Theoretical Plates	9520	> 2000
Tailing Factor	1.15	<1.5

Table8: System suitability parameter

CONCLUSION:

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Saxagliptin 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 Saxagliptin in pure form and its dosage form.

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