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Method Development and Validation of Saxagliptin Hydrochloride by RP-HPLC Method

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ABSTRACT
A sensitive, exact, rapid, avaricious and robust HPLC method was developed for the quantification of Saxagliptin Hydrochloride (SGH) with UV detector. In this method, a reversed-phase Grace C18 (250mm x 4.6ID, Particle size: 5 micron) column with a mobile phase of methanol: water (80:20; v/v) at 0.8ml/min flow rate was used to separate SGH with a detection of 212nm.The volume injected was 20 µL. The retention time of SGH was obtained as 4.196 min. All necessary validation parameters and system suitability tests were carried out in details. The analytical curve was linear (r^2 = 0.999) over a wide concentration range (10 -50 µg/ml). The system shows adequate accuracy with relative standard deviation less than 2.0%. The method showed good duplicability and recovery with % RSD less than 2%. So, the proposed system was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for practice analysis of Saxagliptin Hydrochloride (SGH) in bulk drug.
Keywords: *RP-HPLC estimation, Method development, Validation, Saxagliptin HCl.*

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INTRODUCTION

Analytical chemistry termed as science of determining the components of materials in terms of the elements or compound contained. The approach of this science are used to recognize the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important in nearly all aspects of chemistry. Analytical techniques proved in assuring and maintaining the quality of substance and are critical components of QA and QC.
Analytical method should be,

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Should be fully optimized before transfer for validation of its characteristics such as precision, accuracy, sensitivity etc.
According to USP, system suitability tests are integral part of chromatographic methods. These tests are used to verify that the reproducibility and resolution of the system are adequate for the analysis to be performed. framework such as plate count, symmetry factor, resolution and duplicability (%RSD retention time and area for 6 repetitions) are determined and compared against the specifications set for the method.
Now a day reversed-phase chromatography is the most commonly used disconnection method in HPLC due to its wide application range. It is approximate that over 65% (possibly up to 90%) of all HPLC disconnection are convey out in the reversed phase manner. The reasons for this incorporate the clarity, flexible, and scope of the reversed-phase method as it is able to hold compounds of a various duality and molecular mass.
Reversed phase chromatography has found both logic and preparative appeal in the district of biochemical detachment and clean. Molecules that possess some level of hydrophobic disposition can be separated by reversed phase chromatography with very good recovery and resolution. The separation

device in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. The true nature of the hydrophobic unbreakable interaction itself is a affair of heated discussion but the normal insight assumes the binding interaction to be the result of a favorable entropy effect.

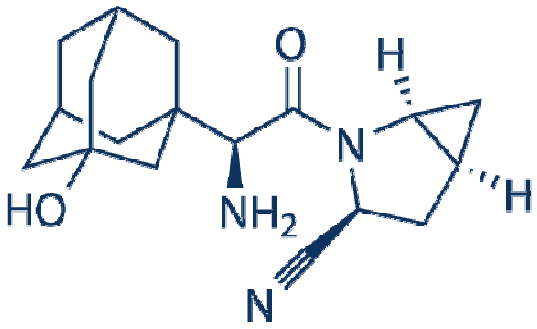

Figure 1: Chemical structure of SAXAGLIPTIN

Saxagliptin is chemically [1S, 3S, 5S-2-2S-2-amino-2-3hydroxy-1adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile, is a new oral hypoglycemic (antidiabetic drug) of the n dipeptidyl peptidase -4[DPP-4] inhibitor class of drugs [1]. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor [2], Saxagliptin slows down the breakdown of incretin hormones, progressively the level of these hormones in the body which is responsible for the advantageous actions of Saxagliptin, including increasing insulin production active in in response to meals and decreasing the amount of glucose that the liver produces. Because incretin hormones are more in response to higher blood sugar levels, the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin.

MATERIAL AND METHODS

Reagents and Chemicals: Water, Acetonitrile, Ortho-phosphoric acid, Methanol, Sodium hydroxide, Hydrogen peroxide (H_2O_2), Tri ethyl amine, and Hydrochloric acid were used in the study.

Chromatographic condition:

A High performance liquid chromatogram equipped with UV-3000-M detector, the purity determination performed on a Grace C18 (250mm x 4.6ID, Particle size: 5 micron) long filled. C18 column the mobile phase consisting of Methanol: Water (80:20).

Preparation of Saxagliptin HCl Standard:

Weighed accurately about 10mg of Saxagliptin HCl standard and transffered into 10mL of volumetric flask, added about 5mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of Saxagliptin HCl 1000ppm).

Further diluted 0.5mL of above stock solution to 10mL of volumetric flask and volume was made up to the mark with diluent (concentration of Saxagliptin HCl 50ppm).

Preparation of stock solution of Saxagliptin:

Weighed accurately about 10mg of Saxagliptin HCl standard and transferred into 10ml of volumetric flask. Added about 5ml of diluent, sonicated to dissolved, cooled and mixed. Solutions of different concentration range as 10%-50% were prepared.

Method evolution: Analytic system development and prove are answer component of any pharmaceutical growth program. HPLC examination method is developed to recognize, amount or purifying combination of attentiveness. This practical short will centre on development and validation activities as put in to drug result. successful method evolution make sure that lab resources are optimized, while methods meet the objectives required at each stage of drug development. procedure validation, required by regulate agencies at certain phase of the drug acceptance process, is explain as the process of reveal Saxagliptin that analytical procedures are acceptable for their intended use.

Validation parameter: The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to the identification, control of impurities and assay procedures is included. Other logical course of action may be considered in later additions to this document. definative validation attribute, which should be considered, are listed below

Linearity.
Accuracy.
Precision.
Limit of Detection (LOD).

Limit of Quantification (LOQ).
System suitability parameter.

Linearity: Linearity is the ability of the analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical Saxagliptin transformation proportional to the concentration Saxagliptin of an analyte in samples within a given span at which the involved response is proportional to the analyte concentration.

Accuracy: Accuracy is the nearness of a measured value to the true or accepted value. Accuracy designate the digression between the convey merit found and the true merit. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analyzed against the standard and blank solutions to ensure that no interference exists.

Precision: The exactness of an logic method is the level of accord among single test results get when the method is pragmatic to many sampling of a similar sample. Precision is a measure of the dependability of the whole analytical process.

Limit of Detection (LOD)

The detection limit of an individual analyte procedure is the lowest amount of analyte which can be detected not necessarily quantified as an exact value. LOD was calculated using the following formula.

LOD = 3.3σ/S

Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity

Limit of Quantification (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte which can be quantitatively determined. LOQ was calculated using the following formula.

LOQ = 10.σ/S

Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity.

System suitability parameter:

System suitability parameter is the evaluation of a composition of an analytical system to show that the performance of the system meets the standard required by the method. This parameter can be calculated experimentally to provide a quantities system suitability test report number of theoretical plates (efficacy) capacity factor, separation (relative retention), resolution, telling factor relative standard deviation (precision).

RESULTS AND DISCUSSION

Determination of λ max

Initially the Ultra violet (U.V) spectrum of Saxagliptin HCl was produced using appropriate U.V spectrophotometer, so as to determine the absorbance maxima or Lambda max (λ max). This is essential since HPLC detection is basically UV based, thus a 20ppm solution of Saxagliptin HCl in water was used to get the following spectra.

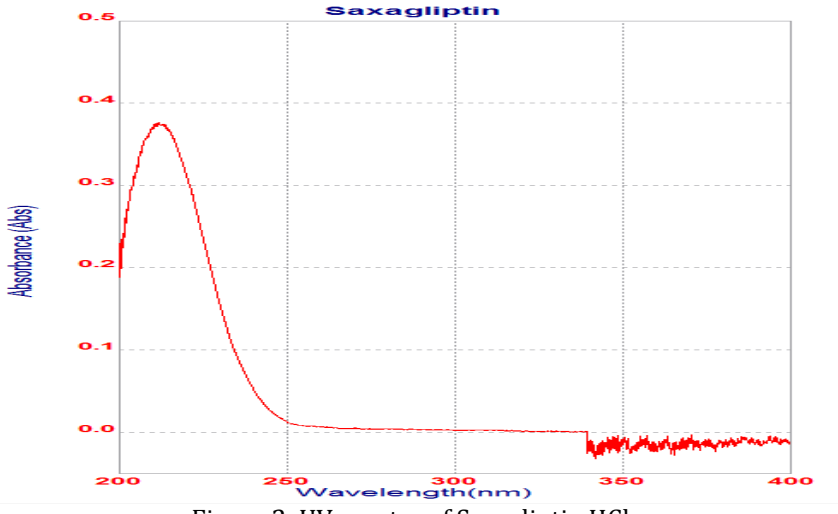

Figure 2: UV spectra of Saxagliptin HCl

Table 1: Chromatographic condition for Saxagliptin drug validation

PARAMETER	CONDITIONS
Stationary Phase (Column)	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: Water (80:20)
Diluent	Methanol : Water (200:100)
Flow rate	0.8ml/min
Injection volume	20 µL
Pump mode	Isocratic
Detector	PDA
Wavelength	212nm
Column Temperature	25°C
Run Time	7.15 min
Retention Time	4.195

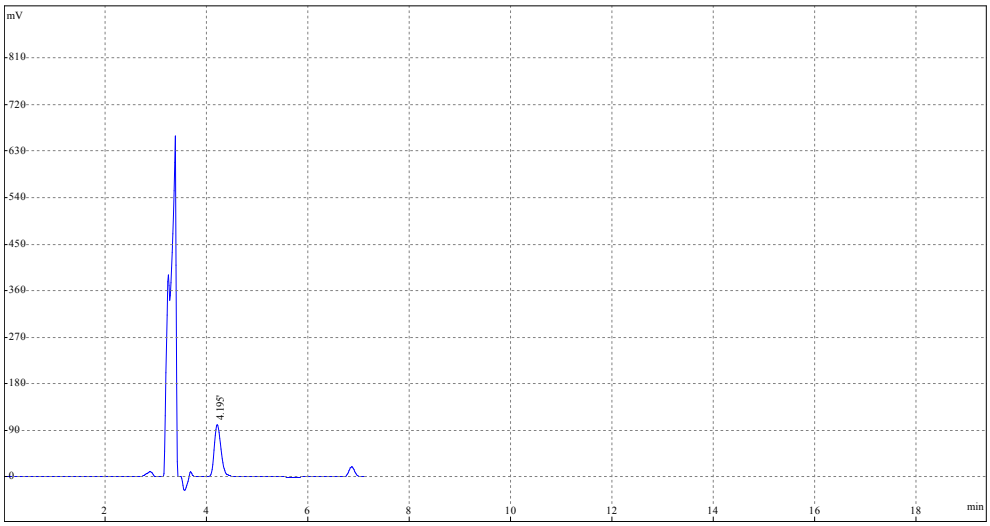

Figure 3: Observed Chromatogram of Saxagliptin

Linearity: For calculating linearity of Saxagliptin HCl a series of standard preparation of Saxagliptin HCl was prepared over a range of 10 to 50% of working concentration level. The linearity graph was plotted from 10 to 50% concentration. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for Saxagliptin hydrochloride was Y= 9832x+10745y with correlation coefficient 0.999 (Figure 3). Where x is the conc. mg/ml and y is the peak area in absorbance unit.

Linearity of Saxagliptin HCl

Table 2: Linearity of Saxagliptin HCl

% Conc. of Standard	Mean Response (Area)	Statistical analysis	
10	**106547**		
20	**209846**	**Correlation**	**0.999**
30	**307489**		
40	**403256**	**Intercept**	**10745**
50	**501469**	**Slope**	**9832.5**

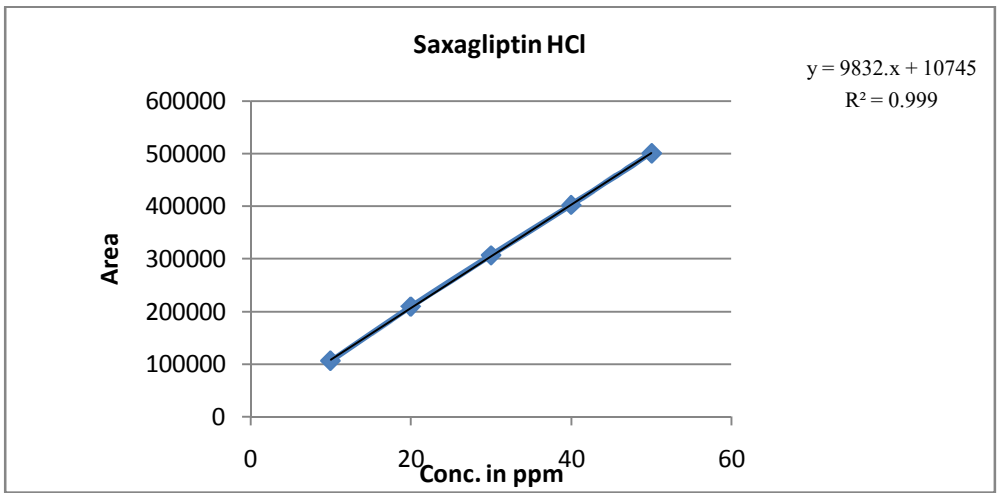

Figure 4: Linearity graph of Saxagliptin HCl

Accuracy:

Recovery of Saxagliptin hydrochloride was determined at three different concentration levels (Table 4).The result indicating that the method was accurate.

Table 3: Accuracy data sheet

Conc.	Area	Mean	SD	%RSD
25	265870			
25	265459	266702	1808.714737	0.678178168
25	268777			
75	1019758			
75	1006574	1012449.667	6707.746591	0.662526426
75	1011017			
125	1612897			
125	1619339	1620590	8388.75396	0.517635797
125	1629534			

Precision:

The exactness of an logic cource of action is usually show as the difference, standard deviation or coefficient of inequality of a sequence of calculation. It can be determined by measuring repeatability, intermediate precision and reproducibility. ICH recommendation recommended that repeatability should be evaluate using a minimum of nine resolution covering the specified scope of the procedure (i.e., three replicates of three concentration). Acceptance criteria is %RSD of the peak area should not be more than 2.0%.

Table 4: Observation of Interday Precision

Day one	Day two
1019758	1004345
1006574	986824
1011017	1006748
Overall Mean	100678
Overall %RSD	1.08%

Table 5: Observation of Intraday Precision

Morning	Evening
1019758	1020586
1006574	995025
1011017	1044221
Overall Mean	1016197
Overall %RSD	1.64

Limit of Detection (LOD)
LOD= 3.3*1808.71437/ 9832.5
LOD= 0.6070 µg/ml
Limit of Quantification (LOQ)
LOQ= 10*1808.71437/ 9832.5
LOQ= 1.8395 µg/ml
System suitability parameters:
The standard solution was prepared by earlier mentioned procedure. After equilibration of column with mobile phase, six replicate injections of 20µL solution were injected. The chromatograms were recorded and peak response i.e. peak area was measured. The % RSD of six replicate injections was found to be not more than 1.5%. The results of system suitability parameters are shown in Table 6.

Table 6: Results of system suitability parameters

Sr. No.	Weight of Standard (mg) Saxagliptin HCl	Area of Standard Saxagliptin HCl
1	10.00	1845245
2		1875496
3		1864987
4		1854789
5		1896547
6		1864789
Mean		1866976
SD		17756.87
%RSD		0.951104

CONCLUSION
It can be concluded from the entire work that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has broad request in different pasture in word of quantitative and qualitative approximation of energetic particle. Final optimization can be performed by changing the temperature, gradient slope, and flow rate as well as the type and conc. of mobile-phase modifiers. The optimized method is validated with various parameters (e.g. accuracy, precision, linearity, detection limit etc.) as per ICH guidelines. The use of the C18 column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor. So the C18 column can be used to achieve high specificity in the shorter time of analysis of Saxagliptin HCl as per ICH Q2 (R2) guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid determination and quantification of Saxagliptin HCl. This developed and validated the method for analysis of Saxagliptin in pharmaceutical preparations is very rapid, accurate, and precise. Moreover, it has advantages of short runtime and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample.

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