



Bioanalytical Method Development and Validation for the Estimation of Saxagliptin in Marketed Tablet Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The concepts, importance and application of bioanalytical method development have been discussed for a long time and validation of bioanalytical methods is widely accepted as pivotal before they are taken into routine use. Now it is widely accepted that bioanalysis is an integral part of the clinical diagnosis, biomarker discovery, pharmacokinetic/ pharmacodynamic characterization of a novel chemical entity from the time of its discovery and during various stages of drug development, leading to its market authorization. Bioanalytical methods, based on a variety of physico-chemical and biological techniques such as chromatography, immunoassay and mass spectrometry, must be validated prior to and during use to give confidence in the results generated. This study describes the development of an innovative, rapid, precise, selective and sensitive reverse phase high-performance liquid chromatography method for the quantitative determination of Saxagliptin (SAXA) in human plasma and pharmaceutical dosage form. Extraction of drug from plasma was done by employing optimized liquid-liquid extraction procedure. The sample was analyzed using methanol: acetonitrile in the ratio of 50:50v/v as mobile phase. Chromatographic separation was achieved on Thermo C₁₈ analytical column (250mm×4.6mm i.d., 5.0µm) as stationary phase using isocratic elution (at a flow rate of 1 ml/min). The peak was detected using UV-PDA detector set at 230 nm and the total time for a chromatographic separation was 20 min. The calibration curve obtained was linear (r² = 0.999) over the concentration range of 5-25µg/ml for SAXA. Method was validated for precision, robustness and recovery.

Keywords: Saxagliptin; human plasma; pharmaceutical dosage form; precision, recovery.

1. INTRODUCTION

A new drug's discovery and development costs over \$1 billion, and it can take up to ten years for the drug to reach the market [1]. Drug discovery and development is the process of creating chemicals and assessing all of their features to see if one novel chemical entity (NCE) can be turned into a safe and effective drug. The strategies used in the drug discovery and development procedures are changing dramatically. For example, pharmacokinetics (PK) is becoming more important in both processes [2,3]. Furthermore, toxicokinetics has established itself as an important component of toxicity testing [4,5]. With the increased emphasis on PK/toxicokinetic analysis and the increased potencies of novel medications, a sensitive and specific bioanalytical technique is required. The emergence of the field of bioanalysis as a critical tool during the process of drug discovery and development is well understood and globally accepted [6-9]. A variety of assays for NCEs, including tests for key metabolites, has been constantly developed over the last few decades to assist various stages of discovery and development [10-14].

Saxagliptin (SAXA) (1S,3S,5S)-2- [(2S)-2-amino-2- (3- hydroxy-1- adamantyl) acetyl]-2 azabicyclo [3.1.0]hexane-3-carbonitrile) is an orally active, potent and selective inhibitor of dipeptidyl peptidase- IV (DPP-4) for the treatment of Type-2 diabetes, is marketed in a fixed dose combination SAXA/ DAPA as tablet in USA, Europe and some other countries [15-16]. DPP-4 inhibitors enhance the body's own ability to control blood glucose by increasing the active levels of incretin hormones in the body. Their mechanism of action is distinct from any existing class of oral glucose-lowering agents. They control elevated blood glucose by triggering pancreatic insulin secretion, suppressing pancreatic glucagon secretion and signaling the

liver to reduce glucose production [17-19]. The structure of SAXA are shown in Fig. 1.

Literature survey also revealed that several analytical methods have been reported for the estimation of SAXA by UV and HPLC [4]. No Bioanalytical HPLC method has been reported for quantitative estimation of SAXA from dosage form and plasma Therefore, it seemed necessary to develop a HPLC method for the estimation of SAXA in tablet dosage form and human plasma. The developed method can be applied successfully to quality control and for other analytical purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1 Material

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

2.2 Reagents and Chemicals

SAXA standard were obtained from Pharmaceutical company as gift sample. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. The 0.45- mm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). HPLC grade water and acetonitrile was used throughout the study. Other chemicals used were of analytical or HPLC grade.

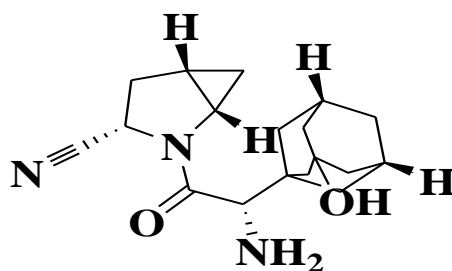


Fig. 1. Chemical Structure of saxagliptin

2.3 Methods

2.3.1 Selection of precipitating agent

Selection of precipitation agent is based on the solubility of drug and good protein precipitation property. Saxagliptin soluble in Acetonitrile and having good precipitating property so the Acetonitrile was selected as the protein precipitation and extraction agent.

2.3.2 Selection of mobile phase

Initially to estimate Saxagliptin number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: acetonitrile in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

2.3.3 Procedure for preparation of mobile phase

Mixed 50 volumes of methanol, 50 volumes of Acetonitrile and adjust the pH 7 with TEA. Filtered through 0.45 μ filter paper.

2.4 Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials Acetonitrile was used as diluents.

2.4.1 Extraction of drug sample

Accurately weighed 10 mg of Saxagliptin was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of plasma, then volume was made up to 50 ml with acetonitrile and vortex it to get complete precipitation of plasma protein. Stand it aside for few minute, precipitate of protein settled down then collect the supernatant layer and add 10 ml of acetonitrile in precipitate to complete removal of drug. Centrifuge the collected supernatant layer at 6000 rpm for 7 min at 4°C and then filtered by whatmann filter paper (no.41). Concentration of Saxagliptin in acetonitrile was 200 μ g/ml. (stock- A).

2.4.2 Preparation of sub stock solution

5 ml of solution was taken from stock-A of Saxagliptin and transferred into 10 ml volumetric

flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 μ g/ml (Stock-B).

2.4.3 Preparation of different solution

0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5 μ g/ml, 10 μ g/ml, 15 μ g/ml, 20 μ g/ml, 25 μ g/ml for drug.

2.5 Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 μ g/ml was prepared. All the solution were filtered through 0.2 μ m membrane filter and injected, chromatograms were recorded at 254 nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

2.6 System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Saxagliptin 10 μ g/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

The physical mixture of Saxagliptin is available in the strength of 10 mg. Based on this different standard solutions were prepared for quantitative analysis, which gives satisfactory results. Stock solution was prepared in the same manner as described in the section. Further dilutions were made to prepare the mixed standard of desired concentration.

2.7 Validation of Developed Method

2.7.1 Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was constructed after analysis of five different (from 5 to 25 μ g/ ml) concentrations and areas for each concentration was recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and

respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

2.7.2 Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

2.7.3 Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

2.7.4 Precision

The precision are established in three differences:

1. Repeatability
2. Intermediate precision
 - a) Day to Day
 - b) Analyst to Analyst
3. Reproducibility

2.8 Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for Saxagliptin indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in Tables-1-4 respectively.

2.9 Intermediate Precision

2.9.1 Day To day precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations. Results of day to

day intermediate precision for Saxagliptin reported.

2.10 Analyst- To- Analyst Precision

Analyst to analyst variation was performed by different analyst in five replicate at five concentrations.

2.10.1 Reproducibility

The reproducibility was performed by chemical to chemical (use of rankem chemicals in place of merck chemicals) variation in five replicate at five concentrations.

2.10.2 Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Methanol: Water (60:40 % V/V), to (55:45 % V/V).

2.11 Detection Limit and Quantitation Limit

The LOD and LOQ of developed method was calculated based on the standard deviation of response and slope of the linearity curve. (Table 1)

2.12 Analysis of Tablet Sample

Tablets amount equal to 10 mg of Saxagliptin was taken in 100ml volumetric flask. This was than dissolve in 13 ml of plasma by sonication for about 10 minutes. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

Table 1. Regression statistics and LOD and LOQ

Parameter	Saxagliptin
Concentration (µg/ml)	5-25
Correlation Coefficient (r ²)*	0.999
Slope (m)*	80.99
Intercept (c)*	19.14
LOD(µg/ml)	0.55
LOQ(µg/ml)	1.50

*value of five replicate

Table 2. Result of precision and robustness

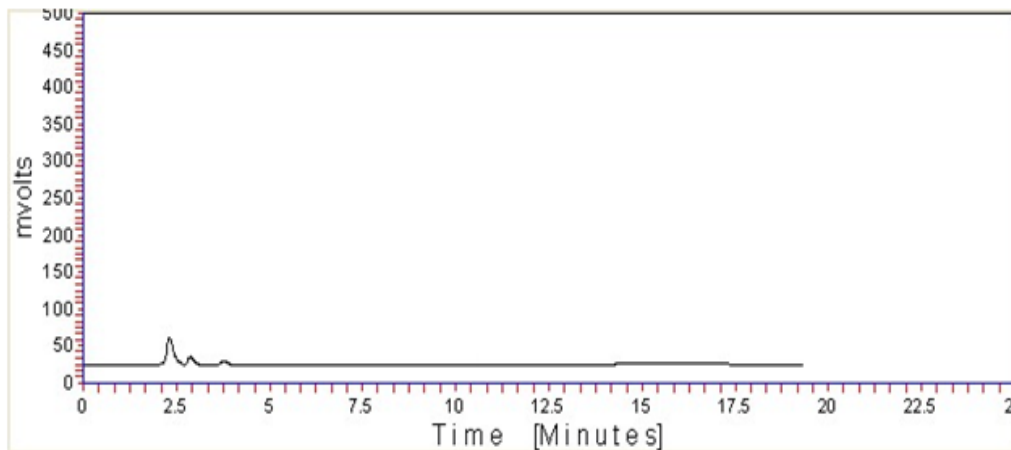
Parameter	% MEAN±SD*
Saxagliptin	
Repeatability	99.447±0.036
Reproducibility	99.172±0.026
Intermediate precision	
Day to day	99.276±0.041
Analyst to Analyst	99.367±0.039
Robustness	99.289±0.060

* Value of five replicate and five concentrations

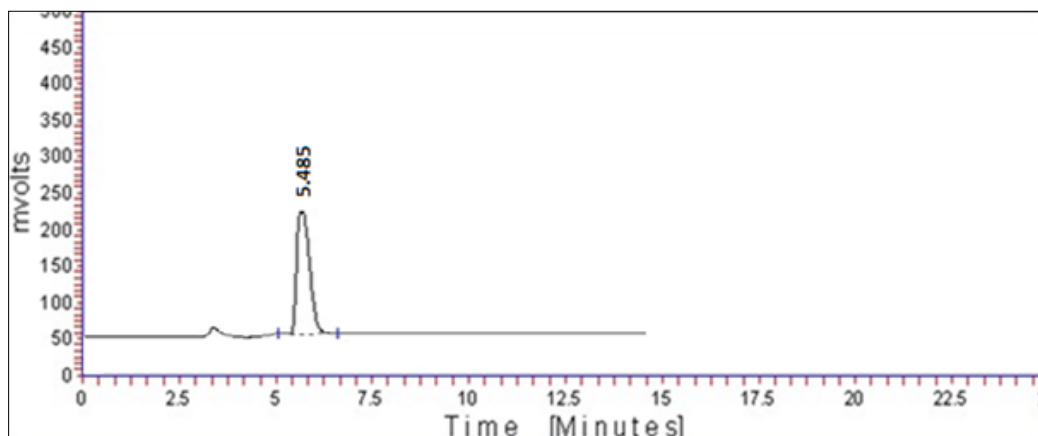
Table 3. Results from recovery studies

% Level	% Mean±SD*
Saxagliptin	
80%	99.51±0.664
100%	99.724±0.234
120%	99.567±0.254

* Value of three replicate and three concentrations



(A)



(B)

Fig. 2. Representative chromatogram of blank plasma (A) and Saxagliptin (B)

Table 4. Analysis of Saxagliptin in marketed formulations

	Saxagliptin *
Label Claim (mg)	5mg
% Found (mg)	4.95
% Assay	99.00
% RSD	0.145

*Average of three determination

3. RESULTS AND DISCUSSION

The method was validated according to ICH guidelines for validation of analytical procedures. The method was validated for the parameters like linearity, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and robustness. The linearity of this method was proved using linear correlation of the peak-area values and appropriate concentrations.

4. CONCLUSION

It was concluded that the present method was fast and easy to perform. Moreover, the method doesn't require various elaborate treatments and tedious extraction procedures. The linearity range, precision, accuracy, robustness, LOD, LOQ and specificity were processed to establish the suitability of the method and the confirmed results were obtained. HPLC has several superiorities compared with UV spectrophotometry, such as smaller detection and quantification limits, small sample volumes and specificity. The proposed method as higher sensitivity than many of the reported methods. Thus, the developed HPLC method is rapid, reliable, and cost-effective and so, it is inexpensive. In addition to the satisfactory, sensitivity and reproducibility as well as the convenience and simplicity and can be proposed for routine analysis laboratories and quality control purposes and are very beneficial for pharmaceutical companies, clinicians and physicians and also can be beneficial for the studies of drug interaction with other combinations.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the

products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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